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Significance of cellulose production by planktonic algae in lacustrine environments.

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SIGNIFICANCE OF CELLULOSE PRODUCTION

BY

PLANKTONIC ALGAE IN LACUSTRINE ENVIRONMENTS

A Dissertation Presented

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Jinnque Rho

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INTRODUCTION

Most, if not all, of the biotic components of an aquatic environment are involved in the complicated series of events associated with the biochemical synthesis and transformation of organic matter. Since much of the non-living organic material present in an aquatic environment may be in the form of plant residues, and since cellulosic materials constitute a significant part of most plant residues, a more complete description of the factors governing the distribution and fate of cellulosic materials in a lacustrine environment should further our understanding of regulatory mechanisms in such ecosystems. For example, if cellulose is a significant fraction of the organic carbon present in water, the activity of cellulolytic bacteria may have a regulatory effect on other microbial populations by supplying, or competing for, growth factors required by those populations.

The phytoplankton play an important role in the production of particulate and dissolved organic matter. It is well known that the presence of cellulosic material in some planktonic algae can be demonstrated by chemical analyses, electron microscopic and X-ray diffraction methods. However, there are no quantitative data available attesting to the production of cellulosic material by planktonic algae in the natural body of water. Furthermore, little or no information is available regarding the source and the fate of such materials in lacustrine environments. Thus this study involved: (1) the

comparison of the numbers and types of phytoplankton present in different aquatic systems with the amounts of cellulosic material present in those systems; (2) the estimation of the cellulose content of selective algal species observed in the water column and the determination of proportion of the cellulose present in the organic seston contributed by the phytoplankton population and (3) the comparison of the vertical distribution of the phytoplankton crop with the cellulose and the related carbohydrate fractions in a relatively unpolluted water body.

REVIEW OF LITERATURE

Organic Matter in Natural Bodies of Water

Organic compounds are present in natural waters in both dissolved and particulate states. These materials may be formed within the body of water as a result of the activities of photosynthetic algae, aquatic plants, and by chemosynthetic bacteria, or they may be formed by agents outside of the basin and enter via water or wind. Hutchinson (1957) divided such compounds into two major categories; the endogenously formed organic matter he designated as "autochthonous" and the exogenously formed material he designated as "allochthonous". He further reported that the autochthonous organic matter varied from one body of water to another according to whether it was produced by phytoplankton or by rooted plants and according to the species involved. He suggested that the allochthonous material originated in either the littoral zone or the headwaters of influent streams.

The quantity of particulate organic matter detected in the surface water of Wisconsin lakes (Birge and Juday, 1922) varied from 0.23-12.0 mg/L with a mean of 1.36 mg/L. Similar results were reported in Lake Michigan (Robertson and Powers, 1965). Birge and Juday (1934) reported that planktonic organisms were the primary sources of particulate and dissolved organic matter in those lakes that received little of their organic matter from outside sources. Tryon and Jackson (1952) found that the amounts of particulate organic matter roughly

paralleled the numbers of phytoplankton present in such system. Microscopic observations have revealed that most of the organic detritus appeared to be of algal origin (Rodina, 1963). However, Duursma (1966) indicated that the concentrations of dissolved organic material appeared to be greater than the amounts that could be associated with standing crops.

On many occasions the ratio of soluble to particulate organic matter was found to be approximately 5.6 to 1 (Birge and Juday, 1934). While, these early studies pointed out that the planktonic organisms were the primary sources of dissolved organic matter in lakes, no attempt was made to determine whether or not the dissolved organic matter detected was the result of the excretion by living organisms or was the result of the disintegration and degradation of dead organisms. Recent investigations indicate that algae liberate extracellular products into surrounding culture media (Lewin, 1956; Nalewajko, 1966), but it appears that the products liberated during the growth make up only a minor proportion of the total dissolved organic matter in natural waters (Fogg and Westlake, 1955; Fogg, 1962).

Lewin (1956) speculated that such compounds served as substrates for the growth of heterotrophic bacteria in the natural situation. However, Duursma (1966) indicated that these compounds do not always play a direct role as microbial nutrients in spite of their relatively high concentrations in natural systems. Rather, he speculated that poly-condensed compounds such as humic acid were formed by bacterial or "enzymatic

processes". He stated that the decomposition and synthetic processes are going on simultaneously. Consequently, the concentrations of many specific compounds are not constant in relation to time even though there are not many dramatic changes in the total amount of organic compounds present in natural bodies of water.

The chemical composition of the organic fraction of natural waters has been studied by Birge and Juday (1922, 1926). They divided the organic material into three arbitrary categories: (1) an ether extract which was determined directly; (2) the crude protein which was calculated by multipling the total nitrogen content by 6.26 and (3) the carbohydrate which was obtained from the residual carbon that could not be accounted for by the ether extract or the crude protein. These investigators found that the particulate organic matter consisted of 37% crude protein, 4% ether extract and 59% carbohydrates. Similar results were reported by Goryunova (1954), who identified half the organic matter present in Lake Beloie as mostly polysaccharides and small amounts of fatty acids.

The compounds identified in, or isolated from, particulate and dissolved organic matter obtained from fresh and marine waters were reviewed by Vallentyne (1957), who listed 45 different compounds present in the particulate material and 15 in the dissolved material. However, Breger (1970) noted that most of the chemical analyses of organic matter present in natural waters were limited to the detection and identification of simple compounds and that complex compounds of higher

molecular weights, which make up about 90% of the total organic matter, were neglected due to the difficulties encountered during their analyses.

The distribution of particulate and dissolved organic matter in the oceans has been studied by several investigators (Menzel and Goering, 1966; Handa, 1970; Menzel and Ryther, 1970). The concentrations of particulate organic matter were found to be highly variable in surface waters and appeared to be directly proportional to the rate of primary production. The distribution of particulate organic matter in waters below 200 meters was remarkably constant as to time, space and depth. It was suggested that the organic materials found below 200 meters were resistant to decomposition and that complete cycles of production and decomposition occurred for the most part at depths above 200 meters. This phenomenon indicated that the consumption of oxygen was probably restricted to areas of high production and that this occurred at some undetermined, but shallow depth.

Seasonal changes in particulate organic matter have been described in large lakes and in small pools and it has been demonstrated that the seasonal fluctuations in the number of phytoplankters paralleled the seasonal trend of particulate organic matter present (Birge and Juday, 1926; Tryon and Jackson, 1952; Robertson and Powers, 1965). However, Moss (1970) studied seston composition in two different pond systems and found that in one pool which was protected from wind and contained few aquatic macrophytes, about a third of the

particulate organic matter was associated with the detritus. A second pool, shallower and more exposed, was entirely colonized by aquatic macrophytes; here, approximately half of the organic matter was tied up in living algae. Moss concluded that "extrapolation of relationship between common seston components from one body of water to another, without a detailed knowledge of the individual limnological features, could be misleading".

Cellulose and Related Carbohydrates in the Natural Bodies of Water

Little or no information is available that deals directly with the cellulose content in natural bodies of water, but there is related information obtained from various investigations on the composition of seston and the origin of its various components. Proximate analyses of particulate matter showed that the carbohydrate fraction was roughly two-thirds of the organic seston (Birge and Juday, 1926). Darnell (1962) stated that this material was apparently derived almost entirely from the biotic component of the aquatic ecosystem. Furthermore, he assumed it to be rich in starch, cellulose, etc., and that it could serve as a major source of carbohydrate for aquatic life if total or partial hydrolysis were to be achieved by bacterial action or other means.

Birge and Juday (1922) were the first to measure carbohydrate fractions present in plankton and other particulate matter in terms of pentosan and crude fiber. They reported that the range detected in net plankton hold from Lake Mendota

was between 1.18% and 5.16% (1.8-36.1 mg/m^3) of total dry weight. The percentage of crude fiber in the organic fraction of the net plankton observed for several years ranged from 2.67% to 20.21% (3.7-67.0 mg/m^3) of the total dry weight. The variation of the pentosan fractions found in the nannoplankton material ranged from 2.84% to 7.61% (42.7-144 mg/m^3) of total dry weight. Although it was not so noted in report, it may be presumed that much of the crude fiber fraction was cellulose. Further, Vallentyne (1957) noted that the carbohydrate fraction reported by Birge and Juday probably included compounds other than those usually classified as carbohydrates.

Monosaccharides occur in living materials mainly as monomers in the more complex polysaccharides and not as free sugars. It has been recommended (Ott et al., 1963) that an effort be made to isolate and characterize the altered carbohydrates present in the particulate fraction. However, because of the difficulty of isolating polysaccharides in pure form, and the difficulty of assessing their purity, the most reliable studies available have been made on low molecular weight carbohydrates either free or liberated by hydrolysis.

Handa (1970) divided the particulate carbohydrates into water soluble and insoluble fractions in order to better understand the vertical distribution of this material in a deep oceanic system. The water soluble carbohydrates were found to account for one-third of the total particulate carbohydrate. Acid hydrolysis of this fraction yielded only D-glucose and periodate oxidation data indicated that this fraction consisted

of a polysaccharide with 1-3 glucan. The water insoluble fraction yielded D-galactose, D-glucose, D-mannose, D-xylose and D-glucuronic acid. The structural investigation of D-glucan, the main components of water insoluble fraction, suggested that this carbohydrate was a 1,2- or 1,4- polysaccharide. The abundance of 1,4-polysaccharides in the cell walls of plants further indicated that this fraction could have been cellulosic material present in cell wall constituents of resident algae. There appeared to be no variation in the monosaccharide composition of this fraction over a range of depths, thus, this fraction was considered highly resistant to biological attack although no assumption was made as to the longevity of this resistance.

Kormondy (1968) observed that about 50% of the dry weight of cellulose was lost in the water column during summer months, and he consistantly found a higher loss at the bottom than at the surface. Other studies (Reynolds et al., 1968; Rho et al., 1968; Segal et al., 1968) indicated that both cellulose and cellulolytic bacteria were present in the water column. Furthermore, these authors reported more cellulose in organic seston than could be accounted for by the observed algal crop. These indirect observations were interpreted as indicating that no cellulolytic activity took place in the water column even though cellulolytic bacteria were present. However, it may be premature to state, as did Handa (1970) that due to the refractory properties of cellulosic materials they settled out of the water column and accumulated on the bottom.

Phytoplankton Composition of Natural Bodies of Water

Regional variations in the numbers and types of algae in water bodies may cause differences in the chemical composition of the particulate matter. Thus, while studies of phytoplankton composition are of importance in understanding the productivity of water mass, they are also necessary when one attempts to obtain quantitative estimates of cellulose and related carbohydrates.

The phytoplankton is composed of cells ranged from less than 1 u to 1 mm. Many suggestions have been made for the classification of the plankton primarily on the basis of size (Round, 1965; Schwoerbel, 1970): (1) the net plankton, or euplankton, which can be caught with fine meshed nets (diameter of the pore 60 u), i.e., Asterionella, Tabellaria, Ceratium, Peridinium, etc.; (2) the nannoplankton, which is made up of cells less than 60 u in diameter, i.e., the flagellates and coccoid Chlorophyta, etc. and (3) the u-algae, which are only a few microns in size and composed of small flagellates and other coccoid Chlorophyta. Comparisons between the total numbers of u-algae and larger algae showed that the former are usually more numerous (Lund, 1959). While the average weight per cell of the larger forms is of the order of ten times that of the u-algae, it can be conservatively estimated that the crops of the larger forms are usually greater than those of the u-algae. To support this, Lund (1962) reported dry weight data of many of the algae which showed that the green u-algae vary

from 3-20 ug per million, and individuals of Myxophyceae from 6-20 mg per million. Similar results were observed in various planktonic algae by Nalewajko (1966).

The seasonal, spacial distribution and occurrence of algae have been related to fundamental factors (Chandler, 1945; Rawson, 1957; Sanders, 1957). Along with many other factors, phytoplankton populations were found to be partly dependent upon the chemical composition of the water mass (Edelstein, 1965; Round, 1965; Sparling and Nalewajko, 1970). Since the chemical nature of the water exerted a selective effect on lake phytoplankton, the species present in dystrophic, oligotrophic and eutrophic waters tended to be characteristic of these respective habitats.

Round (1965) reviewed the algal composition of the communities in different habitats. In small ponds the species were determined by the chemical composition of water. Ponds with neutral to alkaline water, generally rich in nutrients, with sediments composed of decaying organic matter tended to support those members of Chlorococcales which are often regarded as characteristic of small bodies of water. Round further observed that larger ponds often had a more distinct planktonic community, which was not easily separable from that of eutrophic lakes. Several genera of diatoms appeared, e.g., Melosira, Rhizosolenia, Fragilaria, together with blue-greens of the genera, Microcystis, Anabaena, Aphanizomenon, etc.

The composition of the phytoplankton population has been long recognized as corresponding to the trophic status of the

lake. The usual scheme for distinguishing oligotrophic from eutrophic plankton has been tabulated by Rawson (1956) (see Appendix I, Table 1). These criteria have been employed with some success to examine temperate lakes in Europe (Skulberg, 1964), in the Great Lakes and Western Canada (Rawson, 1956; Buzniak and Kennedy, 1968) and southern Ontario Lakes (Sparling and Nalewajko, 1970).

Skulberg (1964) noted that the composition of the phytoplankton in oligotrophic lakes could be correlated with the trophic stages of the lakes. Diatoms and Dinobryon, considered good indicators for oligotrophic conditions, were dominant in oligotrophic lakes, and Myxophyceae and Chlorophyceae made up a considerable part of the phytoplankton in eutrophic lakes. However, Rawson reported that the dominant species of algae in the Great Lakes and in the large oligotrophic lakes of Western Canada were not those commonly regarded as oligotrophic indicators. For example, Dinobryon species were not abundant in large oligotrophic lakes. Further, while the diatoms were distinctly dominant in many lakes, the dominant genera of diatoms were those usually associated with eutrophic conditions. It was also observed that in eutrophic lakes the number of species of Chlorococcales was likely to exceed the number of species of Desmidiaceae, while in oligotrophic lakes the reverse condition was found. Rawson concluded from these observations that the numbers of species of certain groups, present in a plankton sample, would seem to have less ecological significance than the numbers of individuals of the dominant species.

Recently, Sparling and Nalewajko gave details of the phytoplankton and chemical composition of lake waters. They found that all the species observed in southern Ontario Lakes could be associated with the peculiar chemical composition of the lakes in question. Their association diagrams showed several groupings. More species were found in productive "base-rich" conditions than were found in unproductive "base-poor" lakes. Several species usually associated with oligotrophic systems were also observed in productive systems. They explain this apparent discrepancy by suggesting that when nutrient levels were lowered some oligotrophic species occurred in all lakes.

An exhaustive review of the effect of the composition of phytoplankton species in various habitats is not necessary in an attempt to estimate cellulose production in aquatic systems. However, it appears that the numbers of a particular algal species in the water may be much more significant than the species composition of the algal populations.

Evidence for the Occurrence of Cellulose in Algae

Cellulose is present in the higher plants and some algal groups as aggregates of fibrils, or partly crystalline bundles, which consist of parallel chains of β-1,4 linked glucose residues and yield a typical X-ray diffraction pattern.

The chemical nature of different celluloses are indicated to some extent by their solubility in alkali (Ott et al., 1963): (1) α-cellulose is insoluble cell wall material in 17.5% NaOH;

(2) β-cellulose is soluble in such a solution and is precipitated on acidification and (3) γ-cellulose remains in solution on acidification. β and γ celluloses are not considered to be true celluloses in the sense that they contain only polymers of anhydroglucose units and the term cellulose usually is confined to what Ott describes as α-cellulose.

α-Cellulose exists in number of crystalline modifications which give different X-ray diffraction patterns (Green, 1963). Cellulose I is a highly crystalline cellulose which gives a characteristic well defined diffraction pattern and yields only glucose on hydrolysis. The treatment of cellulose with alkali alters the X-ray diffraction pattern and results in the production of cellulose II which has a lower crystallinity than cellulose I. Cellulose III and IV are less characterized and are not known to occur in nature. Kreger (1967) has summarized a series of algal cell wall studies and divided cellulose into three types on the basis of X-ray diffraction patterns. X-ray diagrams give evidence that different cellulosic materials contain the same recurring cellulose unit, but that the physical form of the macromolecule is readily altered and changes may occur in the native cellulose during the pre-extraction of other polysaccharide material from the algae (Percival and McDowell, 1967).

Microfibrillar structures that can be associated with the presence of cellulose have been clearly demonstrated by electron micrographs of the cell walls of a number of algae (Lewin et al., 1951; Northcote et al., 1958). Likewise, X-ray diffraction

patterns (Kreger, 1967; Nieduszynski and Atkins, 1970) indicate the presence of cellulose, but in the absence of chemical evidence, these data must be interpreted with caution.

The celluloses occurring in macroscopic marine algae have been the subject of many investigations due to their widespread occurrence and commercial importance (Percival and McDowell, 1967). Little is known, however, concerning the cellulose content of the epiphytic and planktonic algae which play a dominant role in the vegetation of fresh water. Early studies (Fritsch, 1938)utilizing histochemical staining methods suggested the presence of cellulose in the cell walls of fresh water Chlorophyceae, Dinophyceae, Chrysophyceae and Cyanophyceae. Further evidence was not available to demonstrate whether this substance was built up entirely from glucose residues or that the fundamental β-1,4 linkages were present. Although cellulose is usually thought of as a β-1,4 linked polymer of glucose, a number of studies indicate that cellulose may be a partner in a more complex arrangement.

Chlorophyceae. Among the fresh water filamentous green algae, including Spirogyra (Nicolai and Preston, 1952; Kreger, 1957; Dawes, 1966), Oedogonium (Parker, 1964), Nitella (Hough et al., 1952) and Chara (Amin, 1955a), the cell walls were found to consist of cellulose microfibrils. The main cell wall constituents of Nitella were found to consist largely of cellulose-like polyglucosans, while the Chara cellulose and the Oedogonium cellulose both yielded, on hydrolysis, D-glucose, D-xylose, L-arabinose and uronic acid (Amin, 1955b).

The colonial green algae, Hydrodictyon africanum (Northcote et al., 1960), Scenedesmus quadricauda (Bisalputra and Weier, 1963; Burczyk et al., 1970), Pediastrum tetras and Gonium sp. (Parker, 1964) have been studied. An analysis of the cell wall (39.2% of the total dry weight) of Hydrodictyon showed that it contained α-cellulose (69% of cell wall dry weight) and hemicellulose (16%). The cellulose fraction was reported to be composed of polymers containing glucose and mannose, while the hemicellulose fraction contained glucose, mannose and trace of xylose and arabinose. The structure of the cell walls of Scenedesmus quadricauda, upon observation with electron microscope, appeared to made up of three distinctive layers. The thick inner layer bound the cells of the coenobium together. Pediastrum walls appeared highly crystalline but contained structures these differed greatly from microfibrils of typical cellulose (Parker, 1964). Further, the chemical analysis showed that the crystalline polysaccharide consisted of approximately equal amounts of D-glucose and D-mannose. The cell walls of Hydrodictyon had a similar chemical composition but microfibrils resembling those described by Northcote et al. (1960) were not observed. The cell walls of Gonium revealed no distinct X-ray bands and appeared free of cellulosic material but were composed of mucilaginous substances. Most of these studies contributed information that was more qualitative than quantitative, i.e., the presence or absence of cellulose was ascertained but the amount per cell was not determined.

The cell walls of several unicellular green algae,

including Chlamydomonas (Lewin et al., 1951; Pakhomova and Zaitseva, 1969), Glaucocystis (Schnepf, 1965) and Chlorella (Northcote et al., 1958; Olaitan and Northcote, 1962) were reported to be composed of cellulose. The cell walls of Chlorella pyrenoidosa made up 13.6% of the weight of the whole cell. A quantitative chemical analysis of the walls was made with respect to α-cellulose, hemicellulose, protein, lipid and ash. On hydrolysis the α-cellulose (15.4% of cell wall material) yielded glucose, galactose, arabinose, mannose, xylose and rhamnose, while the hydrolysate of the hemicellulose fraction (31.0%) contained galactose, mannose, arabinose, xylose and rhamnose. Similar chemical components were observed in the cell wall of a flagellate green alga, Chlamydomonas. However, it was determined that different cultural conditions resulted in marked differences in the characteristics of the cells studied and therefore probably in the chemistry of the cell wall (Prince, 1963).

Chrysophyceae. There are no conclusive chemical data available concerning the presence of cellulose in Chrysophyceae even though several early studies have shown its presence by staining methods (Fritsch, 1938). Ricketts (1966) investigated the gross chemical composition of the marine chrysophytes, Chrysochromulina and Prymnesium. The carbohydrate concentrations were variable from 5 to 57% of the cell dry weight, depending upon the state of nutrition of the cells. No attempt was made during this study to establish the presence of cellulose in these algae. However, electron microscopic examinations of the

fresh water alga, Dinobryon, has revealed the presence of microfibrils in the lorica (Karim and Round, 1967; Doge and Crawford, 1970) which suggests the presence of cellulose.

Dinophyceae. Electron microscopic examinations suggested the presence of cellulosic microfibrils in the cell walls of Peridinium (Venkataraman and Mehta, 1961). The main constituents of the walls of the freshly collected dinoflagellate, Peridinium westii, were carbohydrates which represented 95% of the dry weight of the cell mass (Nevo and Sharon, 1969). The major component was found to be a polymer of D-glucose which differed from cellulose in its X-ray diffraction pattern, solubility in Schweitzer reagent and cadoxen. The glucose units were connected by β-1,4 and β-1,3 linkages. It was concluded that the cell wall layer consisted of another structural glucan which differed significantly from cellulose. This study, however, did not establish whether the two types of linkages were present in the same polymer or in different polymeric molecules.

Cyanophyceae. The evidence attesting to the presence of cellulose in blue-greens is far from complete or conclusive. The gross chemical composition of blue-greens has been studied in following algae: Oscillatoria and Nostoc (Hough et al., 1952), Anabaena cylindrica (Brishop et al., 1954; Dunn and Wolk, 1970), Anacystis nidulans (Drews and Meyer, 1964; Drews and Gollwitzer, 1965; Weise et al., 1970), Phormidium faveolarum and Tolypothrix tenuis (Hocht et al., 1965). The chemical composition of Anacystis nidulans contained 23.8% carbohydrate, 27.6% protein

and 36% lipids. The polysaccharide fraction, on hydrolysis, yielded mannose, glucose, and smaller amounts of galactose and fucose.

The chemical composition of heterocystous blue-greens differed from that of unicellular blue-greens. The Anabaena polysaccharide was found to be similar to that of the complex acidic polysaccharides isolated from Nostoc in that it included glucose, mannose, xylose and smaller amounts of fucose, galactose, rhamnose and arabinose. Both polysaccharides showed a marked resistance to acid hydrolysis. After comparative chemical analyses of the walls of vegetative cells, heterocysts, and of the mucilage of Anabaena cylindrica, Dunn and Wolk (1970) found that the walls of vegetative dells were composed of an amino compound with a mannose rich carbohydrate component. The carbohydrate concentrations were varied in the heterocyst wall (62%) and in the spore wall (41%), but carbohydrate moieties were similar: i.e., high in glucose and mannose and low in galactose and xylose. Glucose constituted 75% of the total sugar residues in both heterocyst and spore walls. Mannose was the major carbohydrate moiety in vegetative cell walls. It appeared that the walls were not soluble in Schweitzer reagent and were not digested by cellulase. It can be concluded that this study offered no support for the presence of cellulose in blue-green algae that had been suggested by Fritsch (1938), and Frey-Wyssling and Stecher (1954).

In contrast to the above chemical studies of cell wall material, electron microscopic examination of mucilaginous cell

envelope material from Nostoc has revealed a fine, fibrillar component which Frey-Wyssling and Stecher suggested was cellulose. Further, Lamont (1969) demonstrated a helical orientation of microfibrils in the mucilaginous investiments of Oscillatoria. He proposed that since glucose and mannose residues appear to be major components of some blue-green algal mucilages, it is possible that the microfibrils observed in the mucilages of Oscillatoria and Lyngbya are composed of cellulose. This observation was similar to those obtained with cell wall material of green algae, Hydrodictyon (Northcote et al., 1960) and Pediastrum (Parker, 1964). In those cases it was apparent that polymannose was associated with the cellulose chains in the native walls. Because polymannose chains replaced the cellulose chains in the cell walls of certain red algae (Jones, 1950) and because mannose containing materials appeared to be limited to mucilaginous envelopes, it is difficult to draw conclusions as to the presence or absence of cellulose in the capsular material characteristically surrounding blue-green algae.

It is clear from the foregoing review of the literature that the evidence for the occurrence and distribution of cellulose among algae is far from complete and is not conducive to an understanding of the production and fate of algal cellulose in aquatic environments.

MATERIALS AND METHODS

Sampling Areas and Procedures

Water samples were collected from three different aquatic environments; Quabbin Reservoir, and two small experimental farm ponds.

Quabbin Reservoir is a large man-made lake in central Massachusetts. It has a surface area of 100 km^2, a maximum length of 28.8 km, a maximum depth of 45 m, and the average depth of 27 m. It is so designed that the water flowing into the reservoir is diverted by a series of baffle dams and land masses with the result that the water in the main portion of the reservoir has very little suspended material, and a sparse bacterial population. Sampling was carried out in the main portion of reservoir, which is essentially oligotrophic and having a maximum depth of 25 m. Two sampling stations were chosen for their accessibility and were assumed representative of main body of the reservoir (Fig. 1). Station D represented a relatively deep portion of reservoir which, because the reservoir design, could be assumed to contain primarily particulate matter of endogenous origin. On the other hand, Station H represented an area where much of the particulate matter was of exogenous origin. Samples were obtained during the summer of 1969 and 1971.

Two small experimental farm ponds, located on the campus of University of Massachusetts, Amherst, Massachusetts, were selected because of their apparent differences in trophy.

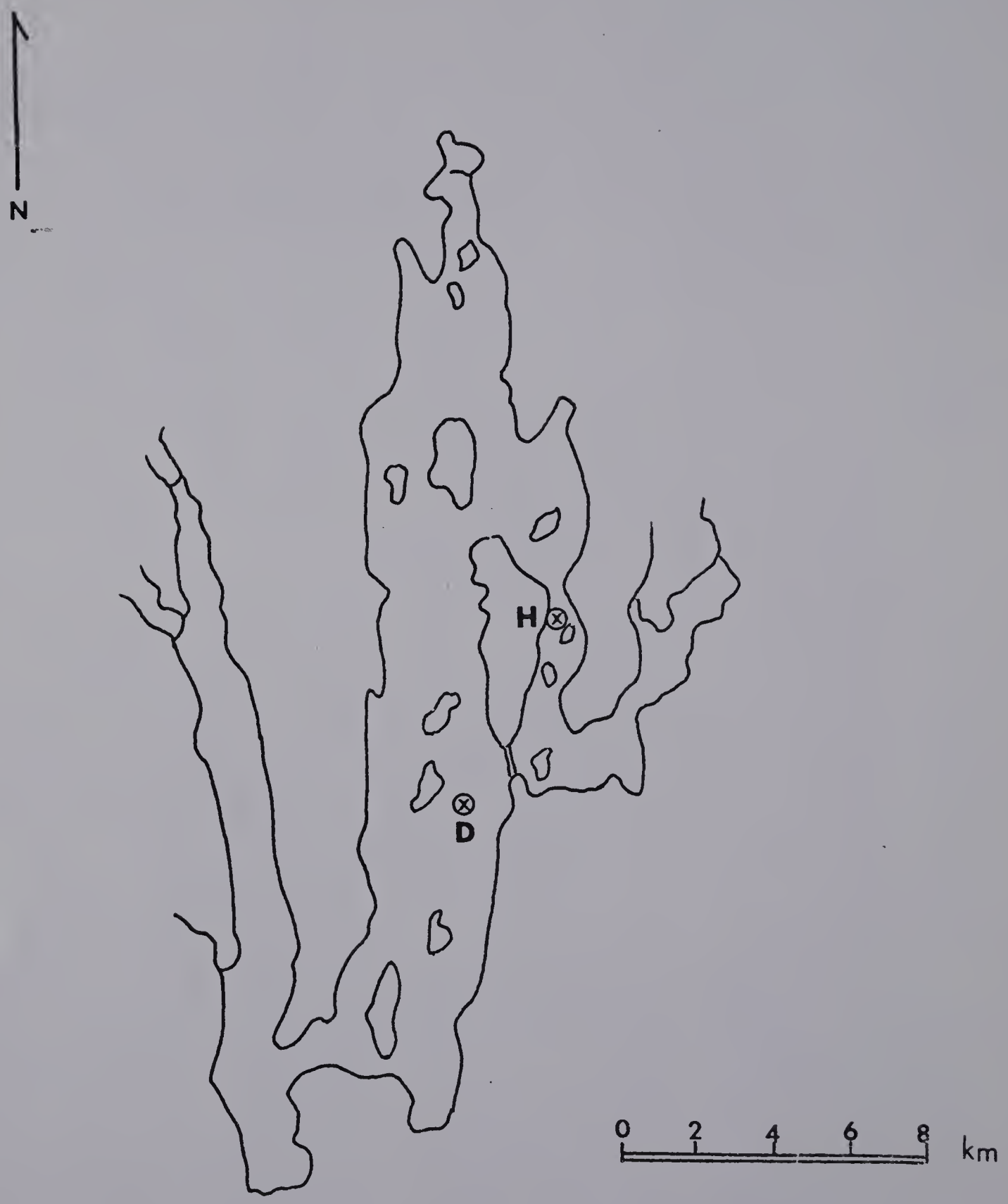


Fig. 1. Map of Quabbin Reservoir showing location of sampling sites.
D; Station D
H; Station H

They were designated as the Upper and Lower Ponds.

The Upper Pond lies in the center of grass land and is surrounded by hills. Its main sources of water are from the fluctuating flow of small streams, surface drainage and precipitation. Its maximum depth is approximately 2 m. The bottom sediment is clay and silt, and water has a yellow-brown coloration. It is protected from wind by its lower location in relation to the surrounded woodland. Aquatic macrophytes are not abundant.

The Lower Pond lies adjacent to a horse grazing area and orchard. It is surrounded by grass and woodland. This pond receives water from small streams, and surface drainage; its constant level is controlled by an outlet drainage system. The influent of water is rich in nutrient salts from the adjacent horse pasture and orchard. Its maximum depth is approximately 2 m. Aquatic macrophytes (a majority belonging to genus Potamogeton) are abundant and appear on the surface within its entire area during summer months. The water is essentially colorless.

Limited chemical and biological data indicate that the Upper Pond is edaphically a dystrophic system and the Lower Pond is an eutrophic system. Both ponds were investigated from January to July, 1971.

Water samples for algal counts and chemical analysis of seston were obtained with a Kemmerer sampler from the deepest portion of the three water bodies. Samples of 5 to 8 liters were obtained routinely; one liter was used for algal counts

and 4 to 6 liters were used for carbohydrate analyses.

For algal determinations, one liter aliquot of Quabbin Reservoir sample was centrifuged in a Foerst Continuous-flow centrifuge and the concentrate transferred to vials and diluted to 10 ml or more depending on the turbidity of the sample. On the other hand, one-half liter of pond sample was filtered through a membrane filter (o.45 u pore size, Millipore Corp.). The particulate matter on the membrane was suspended with 10 ml of distilled water and mixed with a Voltex Mixer. The larger algae were counted using a Sedgewick-Rafter counting cell by the strip method (Am. Public Health Assoc., 1971) and a hemacytometer was employed for smaller algae. The remaining concentrate was used for isolation of predominant species.

Isolation and Culture of Algae

Concentrated water samples were either spread on agar plates (Bold's medium +1% agar) or inoculated into liquid Bold's medium. Inoculated plates and tubes were illuminated under two 40 W cool white fluorescent lights at 20 C for 2-3 weeks. Periodic microscopic examinations were made to determine the presence of predominant or desired species on the plates. Selected clones were streaked on fresh plates. Isolation of the clones, which appeared free of other algal types and fungi, was accomplished by the micropipetting method of Pringsheim (1964). Surface inoculation of agar plates alone was not an ideal method for the isolation of algal types due to the development of many contaminating bacterial colonies and fungal mycelia over the surface of plates. When surface inoculation was used in conjunction

with the micropipetting method, most of the contaminants were eliminated.

Although attempts were made to isolate and to grow the majority of the algae encountered in the water samples, only seven representative species could be cultured under laboratory conditions. Of these, bacteria free cultures of Chlorella sp., Chlorococcum sp., Ankistrodesmus sp., Chlamydomonas sp., and Scenedesmus sp. were grown in mass culture. The mucilaginous Gloeocystis sp. could not be isolated free from bacteria, but unialgal cultures were obtained.

In order to study those genera encountered in the water column, but which could not be isolated in the laboratory, seven representative species were obtained from the Stock Culture Collection of Indiana University. These stock cultures, including Oocystis marssonii, Tetraedron bitridens, Coelastrum microporum, Scenedesmus quadricauda, Staurastrum orbiculare, Cosmarium botrytis and Pediastrum bitradiatum, were obtained and maintained in bacteria free conditions.

The algae used in this study were cultured in 500 ml round bottom pyrex flasks. The cultivation methods used in this investigation were similar to those of Hoogenhout and Amesz (1965) and Komarek and Ruzicka (1969). Axenic cultures of algae were inoculated into the vessels containing 250 ml of liquid culture medium (Bold, 1942). These vessels were placed in a temperature controlled 20±2 C water bath which was constantly illuminated by white fluorescent lamps (4-40 W cool white) on both sides. Aeration and mixing were provided by a constant

flow of sterile air (2% CO_2+filtered air). In some cases growth as a function of pigment production was measured at 680 mu in 1 cm cuvettes with Beckman DU spectrophotometer. In other cases, the number of cells were estimated by microscopic counts using a Sedgewick-Rafter counting cell or a hemacytometer. Assuming that the average pigment content of the cells remained constant during exponential growth, the absorbancy should have been proportional to the number of cells per unit volume, thus the absorbancy measurement could be correlated with the cell counts or dry weight determinations (Hoogenhout and Amesz, 1965).

Growth curves of all species used in this study were determined. Cells in exponential and stationary growth phases were analyzed.

Extraction and Estimation of Cellulose and Related Carbohydrates

The cells were removed aseptically from cultures after various time intervals to determine the growth phase as expressed by absorbance at 680 mu. The aliquots were mixed with distilled water and clumps were dispersed using a magnetic stirrer. After appropriate dilutions, direct cell counts were made using either a Sedgewick-Rafter counting cell for large algae (McAlice, 1971) or a hemacytometer for smaller algae.

Suspensions containing known numbers of cells were passed through preweighed glass fiber filters (984 AH, ultra) which had been prerinsed with distilled water and dried in an oven at 110 C overnight. The extracellular polysaccharides presumably present

in the medium were removed by washing the cells on the filter with distilled water (Lewin, 1956).

The washed cells on the filters were dried in an oven at 110 C overnight and then placed in a desiccator for 12 hours. The weight per cell was calculated from the dry weight of the total cell mass. These residues were analyzed for the various carbohydrate fractions by means of the following procedures.

The cells were digested with distilled water in a steamer at 100 C for 3 hours. The digest was filtered and the filtrate designated as Fraction S. The non-filterable residue designated as Fraction R-1, was washed 6 to 8 times with 10 ml aliquots of distilled water until the pH reached that of the original suspension. Fraction R-1 was then treated with 4% NaOH and refluxed for 3 hours at 100 C. The resulting mixture was filtered as above and this filtrate designated as Fraction H. The residue on the filter, designated Fraction R-2, was washed in the same manner as was Fraction R-1 and then hydrolyzed in 50% H_2SO_4 for 24 hours at 26 C on a rotating shaker, and the resulting mixture filtered. The reducing sugars present in the filtrate (Fraction C), as well as those present in Fraction S and H, were determined colorimetrically by a modification of the method of Dubois et al. (1956). In some cases the sugar content in Fraction C were confirmed colorimetrically by the anthrone reaction (Scott and Melvin, 1953; Updegraff, 1969).

Using the following procedure, reducing sugars were determined. After an appropriate dilution was made, 1 ml of filtrate was pipetted into a test tube. To this, 1 ml of 5% phenol (w/v)

and 5 ml of concentrated sulfuric acid (98% Fisher reagent) were added directly against the liquid surface. After 2 min at room temperature the tubes were completely mixed and placed in a 25 C water bath for 5 min. Optical densities were then measured at 485 mu with a Beckman DU spectrophotometer. These measurements were made employing 1 cm cuvettes and the presumptive fractions of soluble carbohydrate (Fraction S), hemicellulose (Fraction H) and cellulose (Fraction C) were measured against blanks containing H_2O, 4% NaOH and 50% H_2SO_4, respectively. Glucose was used as a standard, and results were presented as glucose equivalents.

In the above determinations Fraction S reflected simple sugars and some starch materials. Fraction H contained hemicellulose primarily. The hydrolysate from Fraction C was assumed to consist of reducing sugars resulting from the degradation of cellulose.

Water samples for carbohydrate analyses were filtered through preweighed and prerinsed glass fiber filter discs (924 AH), which were then rinsed with distilled water, dried, and reweighed. The particulate matter collected from 4 to 6 liters was dried in an oven at 110 C overnight, stored in a desiccator prior to reweighing and the total dry weight of the suspended material was calculated. The extraction and analytical procedures for water samples were basically the same as described for the algal cells.

In order to determine whether the presence of other materials encountered during the analyses might complicate the

interpretation of the data obtained, the absorption spectra of Fraction C from both the algal preparation and the particulate matter of the water column were measured by means of Beckman DB-G grating spectrophotometer.

Extraction and Determination of Chlorophyll

The amounts of chlorophyll detectable vary from species to species and with varying cultural conditions. In these studies, amounts of chlorophylls a and b were measured in order to determine the relationship between the cellulose content and the chlorophylls because there is evidence that the chlorophyll a/b ratio can be related to the physiological state of algal culture studied (Hess and Tolbert, 1967).

Probably because many procedures for the extraction and estimation of chlorophylls have been developed for use with higher plant materials, much difficulty has been encountered in attempt to extract chlorophylls from algae (Hill, 1963). It has been demonstrated that the algal chlorophylls are not usually completely extracted from intact cells with acetone owing to the complex nature of the cell wall. However, when the cell walls were completely broken, reproducible acetone-extraction of chlorophylls could be accomplished. The cell walls of Chlorella have been successfully broken mechanically with Mickle cell disintegrators (Northcote et al., 1958).

For this study a simplified extraction procedure was employed: 10 ml of algal suspensions, containing known numbers of cells, were centrifuged in a clinical centrifuge at 2600

r.p.m. for 3 min. The supernatant fraction was discarded. The cells were resuspended in 5 ml of 80% acetone to which 2 to 4 g of fine glass beads (0.15 mm in diameter) were added. The mixture was placed in a vertical cup of a Mickle cell disintegrator and vibrated for 30 min. After filtration through a glass fiber filters and after appropriate dilution with 80% acetone, optical densities of the filtrates were determined at 645 mu and 663 mu with a Beckman DU spectrophotometer. Chlorophyll concentrations were calculated according to the equation of Hill (1963).

$$\underset{(mg/L)}{Chl.a} = 12.7D_{663} - 2.69D_{645}$$

$$\underset{(mg/L)}{Chl.b} = 22.9D_{645} - 4.68D_{663}$$

Organic Carbon Determination

Organic carbon present was determined as a function of oxidizable particulate organic matter (Hutchinson, 1957; Menzel and Ryther, 1970). It was estimated by wet oxidation method (Strickland and Parsons, 1965) as follows: Suspensions containing known numbers of cells were passes through glass fiber filters and the suspended matter (ca. 5 mg of dry weight) was placed in an acid cleaned 30 ml beaker. Ten ml of sulfuric acid-dichromate oxidant was added and heat was applied for 60 min in a sand bath. The mixture was transferred to a graduated cylinder and brought up to a volume of 50 ml with distilled water. When the solutions had cooled the supernatant was collected by centrifugation. The optical density of the blank solution was measured against the sample at 440 mu. Oxidizable

carbon concentrations were calculated according to the equation of Strickland and Parsons (1965).

$$E = 1.1\ E_{found}$$

$$mg.\ C = E \times F \times V$$

where E = final extinction, F = Correction factor

V = volume of oxidant used.

Glucose was used as a standard, and results were given as glucose equivalents. Determinations of the particulate organic carbon content of the suspended matter were the same as described above.

RESULTS

Composition of Phytoplankton

The relative abundance and distribution of phytoplankton in the three areas studied are shown in Table 1, 2 and 3. Members of Chlorophyceae were the most abundant of the cellulose-contributing algae in terms of numbers and types. Green coccoid cells, the majority of which belong to the genera Chlorella and Oocystis, made up the greatest percentage of the total phytoplankters encountered. Significant numbers of flagellate Chrysophyceae and Dinophyceae were observed in the three systems at different times. These three groups are major cellulose-producers in the bodies of water studied. In addition, it is noted that the total counts of smaller u-algae were higher than were those for larger algae.

Short term observation of phytoplankton populations in the eutrophic Lower Pond ranged from 43,600 to 738,000 units/L. Approximately 70-95% of total phytoplankton observed were cellulose-producers. High algal counts for all species were to be expected in this pond, enriched in nutrients by land drainage from surrounding fields. In dystrophic Upper Pond, the total phytoplankton ranged from 4,500 to 140,000 units/L. Smaller cellulose-producers represented 30-86% of the total phytoplankton populations. Larger cellulose producers appeared only during summer months. The phytoplankters were less abundant in oligotrophic Quabbin Reservoir than in the ponds. Clearly, cellulose-producing algae are present in all of systems in larger numbers

Table 1. Relative occurrence of phytoplankton genera in Lower Pond, expressed as a percentage of total count in units per liter.

Sampling month	Jan	Feb	Mar	Apr	May	Jun	Jul
Standing crop [a]	117	43.6	56.4	128	385	738	521
Group A							
Ankistrodesmus sp.				3.1		2.7	0.6
Chlamydomonas sp.	12.8	9.2	15.6	1.6	1.0	8.1	1.9
Chlorococcum sp.					7.8	0.8	
Cosmarium sp.							18.4
Pediastrum sp.					0.5	0.3	0.3
Scenedesmus sp.	5.6	9.2	0.8	12.5	19.7	13.6	14.8
Staurastrum sp.				3.1			1.2
Tetraedron sp.						0.3	1.6
Green coccoid algae	49.4	52.7	34.7	39.1	39.0	54.2	
Group B							
Ceratium sp.					0.3	1.1	8.8
Dinobryon sp.	6.0	4.6	9.9	1.6	4.4		
Golenkinia sp.					5.2	4.9	27.1
Gymnodinium sp.	19.0	16.5	14.9	5.5	16.4	4.6	19.2
Green filamentous algae				3.1	0.5		
Group C							
Blue-greens [b]				3.9		0.5	1.9
Diatoms [c]	7.2	7.8	24.1	26.5	5.2	8.9	2.0
Group D [d]							2.2

[a] Total count (x 10^3 units/L)

[b] Anacystis sp., Oscillatoria sp.

[c] Tabellaria sp., Asterionella sp., Fragilaria sp.

[d] Euglenoids and unidentified algae

Table 2. Relative occurrence of phytoplankton genera in Upper Pond, expressed as a percentage of total count in units per liter.

Sampling month	Jan	Feb	Mar	Apr	May	Jun	Jul
Standing crop [a]	4.5	15.0	5.0	81.6	26.0	140.0	118.0
Group A							
Ankistrodesmus sp.						2.9	
Chlamydomonas sp.						4.6	
Chlorococcum sp.							16.9
Coelastrum sp.							3.4
Gloeocystis sp.						0.6	
Pediastrum sp.							13.6
Scenedesmus sp.			10.0		7.7		
Staurastrum sp.				1.0		1.1	1.7
Tetraedron sp.							4.2
Green coccoid algae	55.6	33.3	30.0	85.8	50.0	57.1	47.6
Group B							
Closterium sp.			20.0				
Dinobryon sp.				3.4	7.7	13.7	
Gymnodinium sp.						2.3	
Group C							
Blue-greens [b]							0.8
Diatoms [c]	44.4	66.7	40.0	9.8	34.6	17.1	11.0
Group D [d]						0.6	0.8

[a] Total count (x 10^3 units/L)

[b] Anacystis sp.

[c] Fragilaria sp., Tabellaria sp., Asterionella sp., Navicula sp.

[d] Euglenoids and unidentified algae

Table 3. Relative occurrence of phytoplankton genera in Station D, expressed as a percentage of total count in units per liter.

Sampling month	Feb		Jun	
Depth (m)	1	8	1	8
Standing crop [a]	10.2	11.4	37.2	29.6
Group A				
Ankistrodesmus sp.	19.5	6.9		
Chlorococcum sp.			9.7	29.5
Cosmarium sp.				4.1
Gloeocystis sp.			6.3	6.8
Scenedesmus sp.	2.0		2.2	5.4
Staurastrum sp.	7.8	5.3	7.5	8.1
Green coccoid algae	21.6	36.8	6.5	4.1
Group B				
Ceratium sp.				2.7
Dinobryon sp.	31.4	12.3		
Golenkinia sp.			1.1	
Peridinium sp.	9.8	12.3		1.4
Group C				
Blue-greens [b]	3.9		57.0	6.8
Diatoms [c]	2.0	14.1	9.7	23.0
Group D [d]	2.0	12.3		8.1

[a] Total count (x 10^3 units/L)

[b] Anabaena sp.

[c] Cyclotella sp., Asterionella sp., Tabellaria sp., Navicula sp.

[d] Euglenoids and unidentified algae

than is generally assumed.

Those algae which were quantitatively analyzed in order to estimate their cellulose content were arbitrarily divided into four groups. Group A consisted of genera which were cultivated under laboratory conditions and subsequently analyzed to determine the quantity of cellulose fraction per cell. Group B included those genera which could not be analyzed for cellulose, although other studies have suggested that these genera may contain cellulose. Group C included those algae which were believed to contain no cellulose. Group D included those algae which were difficult to identify or did not contain a cellulose fraction in the cell wall.

The presence of several other genera, e.g., Spirogyra, Zygnema, Micrasterias, etc., was observed, but only when large volumes of samples were taken. Hence, these genera were not considered in this study. Attention was concentrated on Group A and B whose members appeared to be the major cellulose-producers in the water column. Fully two thirds of the total number of phytoplankton present belonged to these two groups.

Laboratory Cultivation of Selected Algae

Representative algal isolates were cultured under laboratory conditions. Fig. 2 illustrates a typical growth curve of the unicellular green alga, Oocystis marssonii, measured by both absorbancy and microscopic cell counts. It is clear that the two methods yield nearly identical results. Growth curves of other algae studied are shown in Figs. 3-5. From these, it can

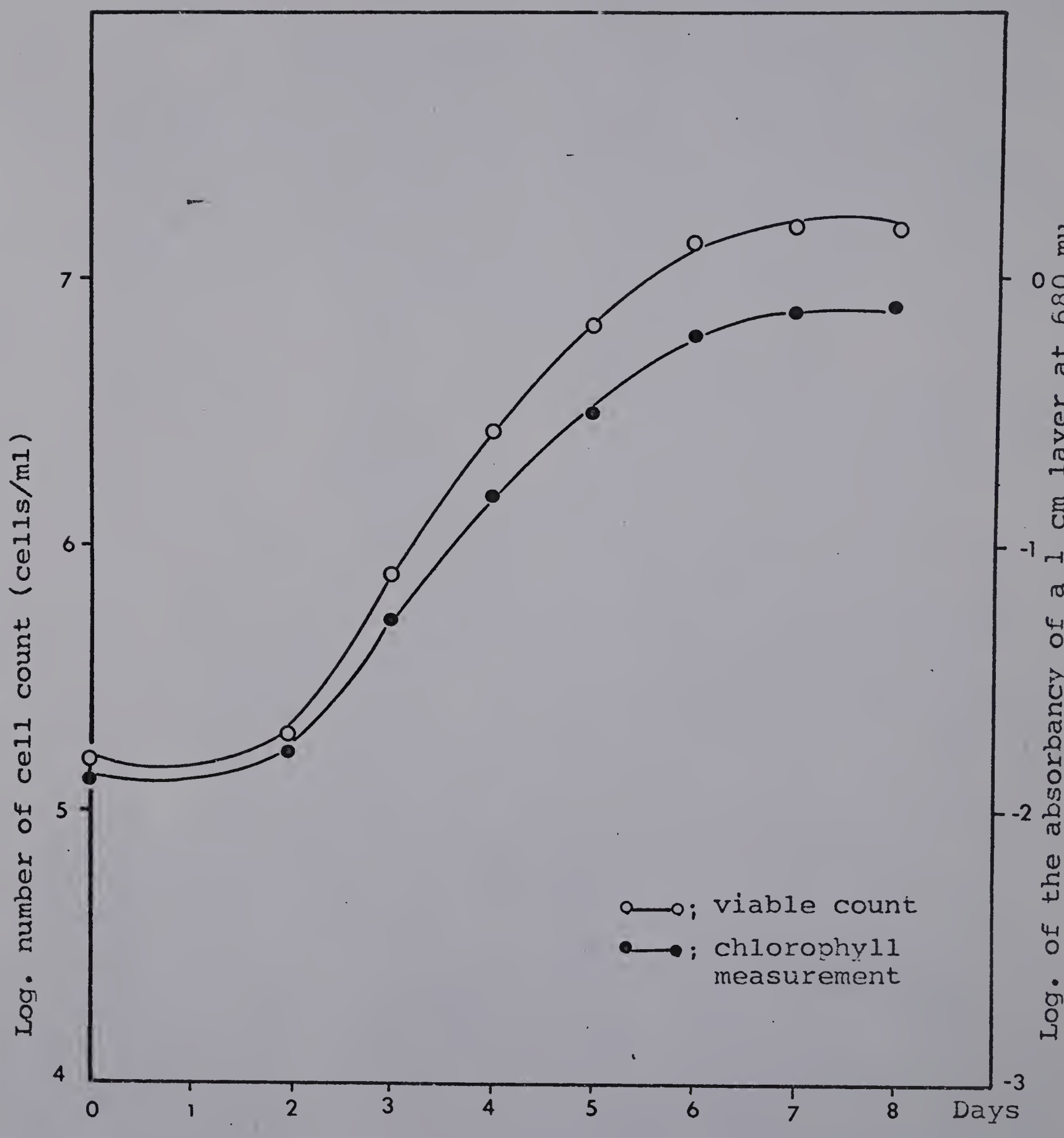


Fig. 2. Growth of Oocystis marssonii in Bold's medium at 20 C and 450 ft-c.

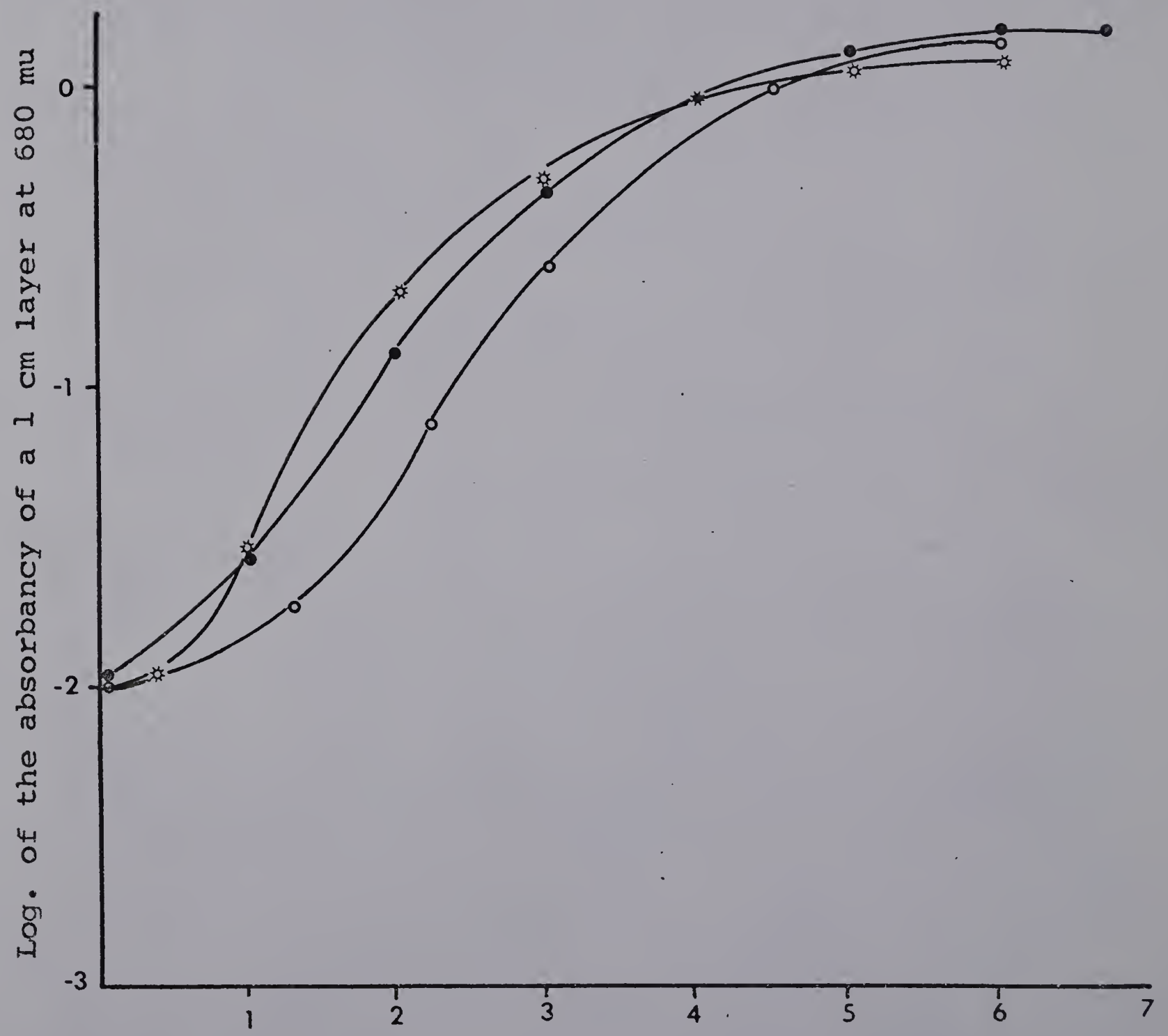


Fig. 3. Growth of Chlorella sp. (✡——✡); Scenedesmus sp. (L) (o——o); and Gloeocystis sp. (●——●) in Bold's medium at 20 C and 450 ft-c.

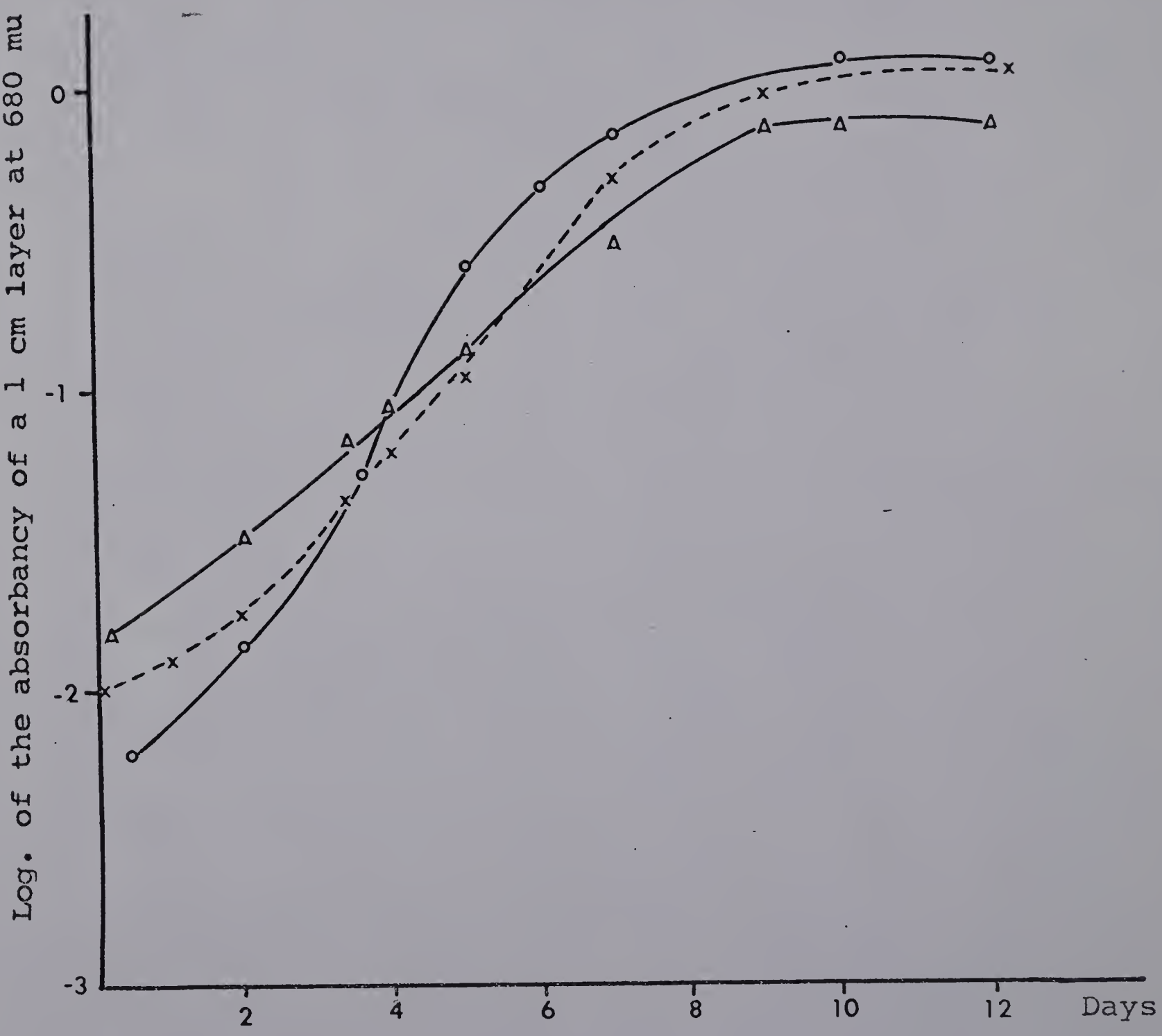


Fig. 4. Growth of Scenedesmus sp. (Q) (o——o); Chlorococcum sp. (x——x); and Ankistrodesmus sp. (Δ——Δ) at 20 C and 450 ft-c.

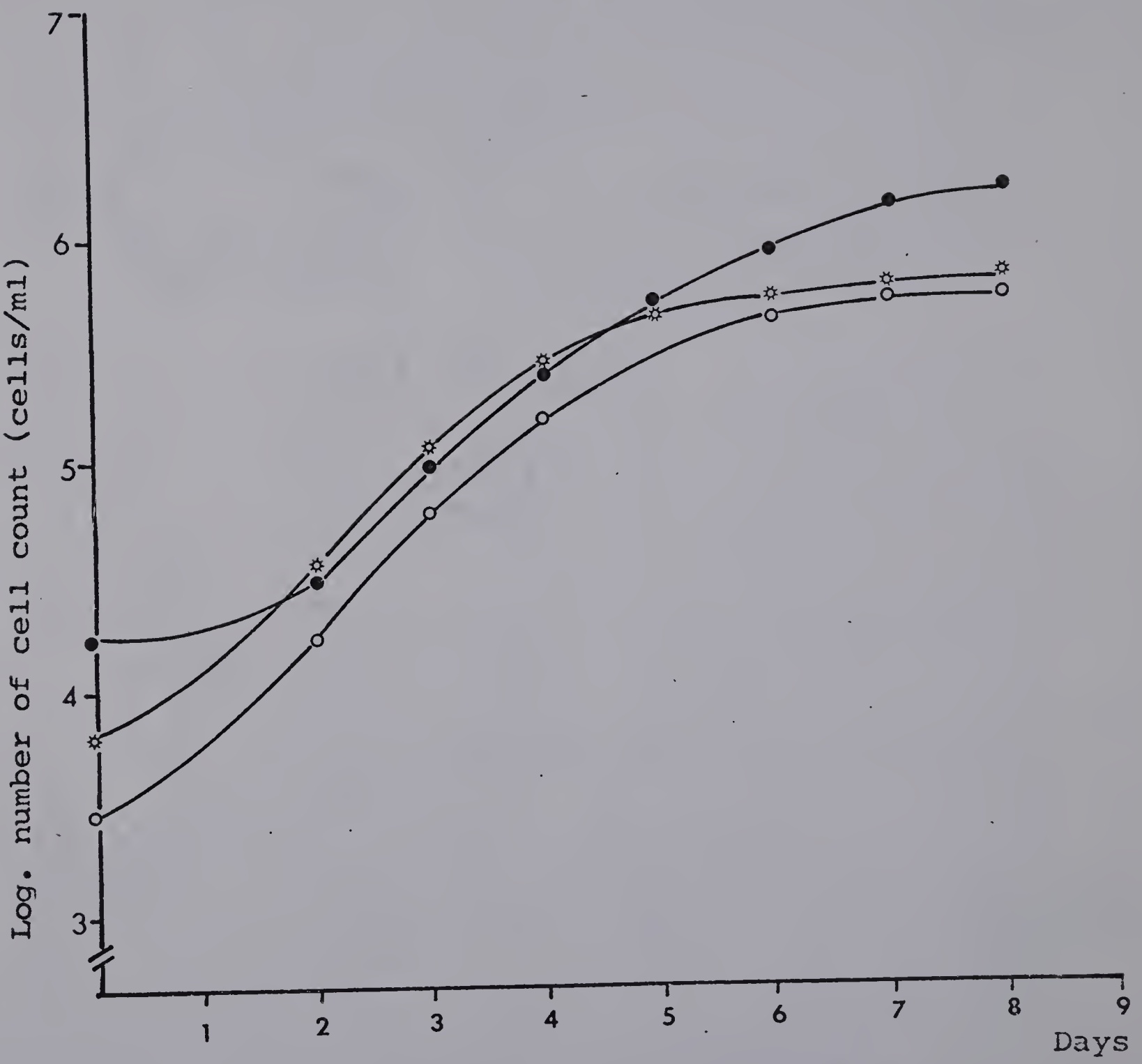


Fig. 5. Growth of Coelastrum microporum (●—●); Chlamydomonas sp.(o—o); Scenedesmus quadricauda (✲—✲) in Bold's medium at 20 C and 450 ft-c.

be seen that, when provided the same conditions, smaller algae e.g., Chlorella and Oocystis, have faster growth rates than the larger forms, e.g., Chlorococcum and Coelastrum. It is also apparent that smaller coccoid algae have shorter generation times than the larger delicate algae.

Prior to cellulose analyses of different cell stages, the chlorophyll content was determined. The ratios of chlorophyll a to b were measured to determine the influence of selected environmental conditions on growth, because some workers have noted that chlorophyll contents are subject to influence by such factors as temperature, exposed light intensity, nutrients, and cell age (Bogorad, 1962; Hess and Tolbert, 1967). The results obtained here were consistent with those usually derived from observations of leaves of higher plants, and algae cultured under laboratory-controlled conditions.

Yield of Polysaccharides from Laboratory Grown Algal Cells

The polysaccharide fractions extracted from laboratory grown algal cultures are presented in Tables 4-6. It is apparent that the average cellulose contents per cell were not constant: variation of about four orders of magnitude were found, depending upon the growth phase studied. Most of the unicellular green algae, except Chlorella sp., showed a relatively higher cellulose content per cell during the exponential growth phase than in the stationary phase. However, the cellulose contents of colonial forms appeared to be greater in the stationary phase than in the exponential phase. This

Table 4. Laboratory analyses of various planktonic unicellular green algae representative of the systems studied.

Species	Growth phase [a]	Chl.a/b ratio	Dry wt. (ug/10^6cell)	Oxidizable carbon (ug/10^6cell)	Carbohydrate fraction [b] (ug/10^6cell)		
					S	H	C
Chlorella sp.	E	1.57	5.79	2.73	0.27	0.73	0.09
	S	1.17	4.09	2.19	0.27	1.36	0.17
Ankistrodesmus sp.	E	1.53	29.81	15.71	1.14	2.56	5.48
	S	1.17	34.06	16.50	1.51	2.92	5.07
Oocystis marssonii	E	1.93	53.85	--	4.07	4.77	9.81
	S	1.83	53.57	28.60	4.17	6.27	6.61
Gloeocystis sp.	E	3.02	120.00	59.08	20.50	1.14	11.98
	S	2.95	85.00	40.10	22.22	6.68	7.58
Chlamydomonas sp.	E	2.19	169.20	53.00	11.68	36.30	11.72
	S	1.89	169.70	91.80	28.10	56.50	9.36
Chlorococcum sp.	E	1.20	391.00	171.20	32.86	26.38	70.60
	S	2.26	177.00	85.00	23.47	9.50	44.40
Tetraedron bitridens	E	1.52	517.40	167.70	37.60	55.40	64.13
	S	1.46	303.70	121.60	16.20	29.40	36.30

[a] E; exponential
S; stationary

[b] S; water soluble carbohydrate- free sugar and some starch
H; water insoluble but alkali soluble carbohydrate- hemicellulose
C; water and alkali insoluble carbohydrate- cellulose

Table 5. Laboratory analyses of various colonial green algae representative of the systems studied.

Species	Growth phase [a]	Chl.a/b ratio	Dry wt. (ug/10^6cell)	Oxidizable carbon (ug/10^6cell)	Carbohydrate fraction (ug/10^6cell)		
					S	H	C
Coelastrum microporum	E	2.33	132.00	41.00	5.11	9.44	22.95
	S	1.81	78.20	43.90	3.31	8.62	26.61
Scenedesmus quadricauda [b]	E	1.54	324.70	176.80	6.30	14.12	62.90
	S	2.60	500.00	220.50	8.10	55.30	197.00
Scenedesmus sp. (Q) [b]	E	2.18	178.30	56.80	4.30	9.20	20.90
	S	1.44	247.30	130.20	6.80	30.00	88.80
Scenedesmus sp. (L)	E	1.12	88.90	52.90	2.40	4.50	12.60
	S	0.82	81.30	45.90	5.70	11.40	18.70

[a] E; exponential
S; stationary

[b] Values based on 1 coenobium of 4 cells

Table 6. Laboratory analyses of 14 day cultures of larger planktonic algae representative of the systems studied.

Species	Chl.a/b ratio	Dry wt. (ug/10^6cell)	Oxidizable carbon (ug/10^6cell)	Carbohydrate fraction (ug/10^6cell)		
				S	H	C
Staurastrum orbiculare	2.52	1,458	671	124.6	117.3	289.8
Pediastrum bitradiatum [a]	1.98	15,714	--	589.3	1,379.5	870.5
Cosmarium botrytis	2.59	9,722	4,300	546.5	547.9	1,766.7

[a] Values based on 1 coenobium of several cells

Table 7. Relative occurrence of carbohydrate fractions expressed as percent dry weight in laboratory cultivated algae.

Species	Growth phase	Carbohydrate fraction S	H	C
Chlorella sp.	E	4.6	12.1	1.5
	S	6.5	33.3	4.2
Ankistrodesmus sp.	E	3.8	8.6	18.3
	S	4.4	8.6	14.8
Oocystis marssonii	E	7.6	8.9	18.2
	S	7.8	11.7	12.3
Gloeocystis sp.	E	17.1	5.1	9.9
	S	26.1	7.8	8.9
Chlamydomonas sp.	E	6.9	21.5	6.9
	S	16.6	33.3	5.5
Chlorococcum sp.	E	8.4	6.7	18.1
	S	13.2	5.4	25.0
Tetraedron bitridens	E	7.3	10.7	12.4
	S	5.3	9.7	11.9
Coelastrum microporum	E	3.9	7.1	17.3
	S	4.2	11.0	34.0
Scenedesmus quadricauda	E	2.0	4.4	19.3
	S	1.6	11.0	39.4
Scenedesmus sp. (Q)	E	2.4	5.1	11.7
	S	2.7	12.1	35.9
Scenedesmus sp. (L)	E	2.8	5.0	14.1
	S	6.9	13.9	22.9
Staurastrum orbiculare	S	8.5	8.0	19.1
Pediastrum bitradiatum	S	3.8	8.8	5.5
Cosmarium botrytis	S	5.6	5.6	18.2

may be due to the unavoidable inclusion of certain dead cell fractions, such as the remains of burst mother cell walls.

A comparison between the cellulose content of the average unicellular green u-algae, with that of the larger desmids indicates that levels for the desmids are approximately 10 to 1,000 times greater than for the green algae. These differences in the cellulose contents are similar to differences in algal dry weights. The cellulose content of the smaller greens varied from 0.1-10 ug/10^6 cells, the larger unicellular and colonial greens varied 10-70 ug/10^6 cells and desmids varied 290-1,800 ug/10^6 cells. For all species studied here the percent dry weight of cellulose varied from 2 to 19% of the total dry weight in exponential growth, and 4 to 39% in stationary growth (Table 7). Higher values were obtained with colonial forms. Lower values were observed when smaller unicellular algae were studied. However, this correlation between algal dry weight and cellulose contents was not considered meaningful because of inconsistencies in the ratios. Yet it is clear that the levels of cellulose in the larger forms are usually greater than those of the smaller algae. Thus, the cellulose contents appear to be dependent upon the species of algae and their stages of growth.

Yield of Polysaccharides from Organic Seston

Amounts of particulate matter in the water column (Quabbin Reservoir) ranged from 0.3 to 1.5 mg/L at Station D (Table 8; Appendix II, Table 2) and 0.8 to 22.7 mg/L at Station H (Appendix II, Table 3). Cellulose constituted 4 to 50% of the total dry weight of the particulate matter encountered at

Table 8. Comparison of oxidizable carbon in particulate matter and particulate carbohydrate fractions of the study areas.

Sampling month	Particulate matter (PM) (mg/L)	Oxidizable carbon (ug/L)	Carbohydrate fraction (ug/L)				Cellulose containing phytoplankton (units/L)
			S	H	C	C as % dry wt.(PM)	
Lower Pond							
Jan	2.4	241	80	85	362	15.0	108,600
Feb	2.4	641	67	70	307	12.8	40.200
Mar	2.1	307	88	37	230	10.9	42,800
Apr	1.5	403	74	38	250	16.7	89,000
May	2.6	1,006	140	123	373	14.4	365,000
Jun	5.6	1,966	230	240	560	10.0	668,600
Jul	5.2	1,522	158	206	553	10.6	489,200
Upper Pond							
Jan	2.8	243	52	165	450	16.1	2,500
Feb	2.3	166	25	60	370	16.8	5,000
Mar	2.3	416	22	50	317	13.7	3,000
Apr	1.1	416	46	39	250	22.7	73,600
May	2.0	457	67	83	178	8.9	17,000
Jun	3.1	1,048	108	137	248	8.0	115,000
Jul	4.4	1,422	70	81	196	4.5	103,100

Table 8. continued

Sampling month		Particulate matter (PM) (mg/L)	Oxidizable carbon (ug/L)	Carbohydrate fraction (ug/L)				Cellulose-containing phytoplankton (units/L)
				S	H	C	C as % dry wt.	
Station D								
Feb	1 m	1.4	162	34	36	192	13.7	9,400
	8 m	1.2	140	36	24	180	15.0	8,300
Jun	1 m	1.2	349	30	16	132	11.0	12,400
	8 m	1.5	393	43	32	144	9.6	11,200

Quabbin Reservoir. The low values were obtained from bottom samples of Station H and the high values were from Station D. The cellulosic material in Station D derived principally from endogenous materials consisting primarily of phytoplankton remains. However, the cellulosic material in Station H appeared to be derived from outside sources other than phytoplankton.

Particulate matter values of Lower and Upper Ponds ranged from 1.1 to 5.6 mg/L during sampling periods (Table 8). The amounts of cellulose encountered in the ponds ranged from 5 to 23% of the dry weight of the particulate matter. The actual cellulose values ranged from 132 to 280 ug/L at Station D; 115 to 1,186 ug/L at Station H; 230 to 560 ug/L in the Lower Pond; and 178 to 450 ug/L in the Upper Pond. High cellulose contents were observed when the phytoplankton population was high in the eutrophic pond, but no relations between cellulose content and phytoplankton population were observed in dystrophic and oligotrophic ponds.

The ratios of oxidizable carbon to particulate matter showed greater variations depending upon the phytoplankton crops present. The concentration of particulate matter increased rapidly when the phytoplankton crop increased during summer months. This indicated that living phytoplankton were the major source of organic material in the particulate matter. Short term observations carried out on the different trophic systems substantiate the above and are compared in Table 8.

Source of Cellulose in Particulate Matter

A high relative distribution of cellulose-producers has

already been demonstrated, as has the fact that these species have high cellulose contents. Here emphasis is placed on the absorption spectra of C-fraction from which the source of particulate cellulose can be ascertained. The addition of this data clarifies the relationships between cellulose-producers and their cellulose contents, and the overall level of cellulose in the system. Thus, our notions about the origin and presence of cellulose in aquatic milieus may be clarified.

The absorption spectra of D-glucose and hydrolysates of purified commercial celluloses together with several other absorption spectra of cellulose fractions extracted from algal cells, higher plants, and particulate matter are shown in Figs. 6-9. All the fractions were similar to that of glucose in that they exhibited maximum absorption at 485 mu. However, C-fractions from algal cells and particulate matter exhibited different absorption patterns in the range 400-500 mu. Since the absorption peak of chlorophylls was observed in the range of 400-450 mu, it is possible that degraded chlorophylls accounted for the second peaks in the algal cell materials and particulate matter.

Seasonal variations of the absorption spectra of C-fractions from particulate matter are shown in Figs. 8 and 9. The diagnostic peaks of glucose appeared to be masked with a second peak in C-fractions extracted from the Upper Pond. However, the appearance of the diagnostic peak coincides with the disappearance of the second peak in C-fractions extracted from the Lower Pond. The diagnostic peaks appeared when the algal

Fig. 6. Absorption spectra of glucose and reducing materials extracted from commercially purified cellulose and higher plants.

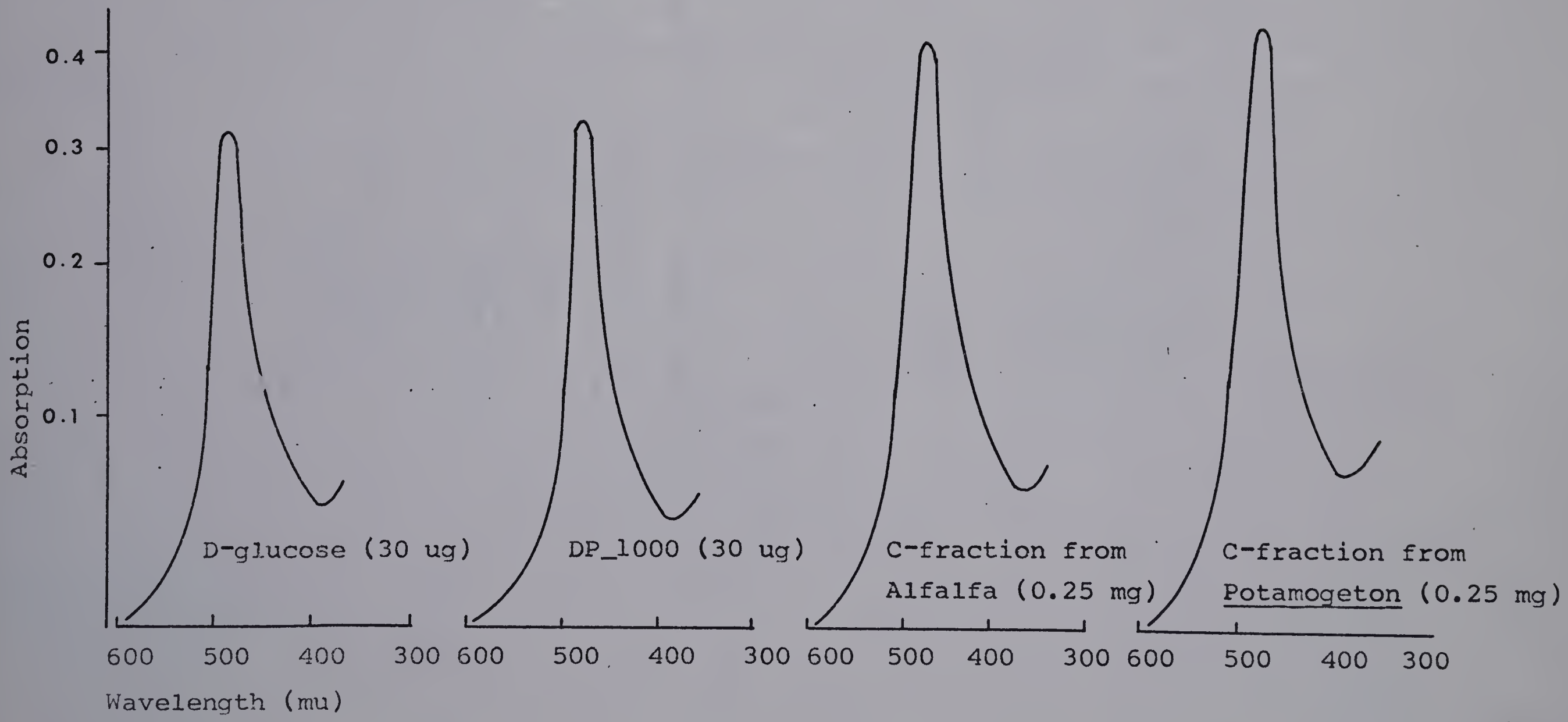


Fig. 7. Absorption spectra of Fraction C extracted from various algae.

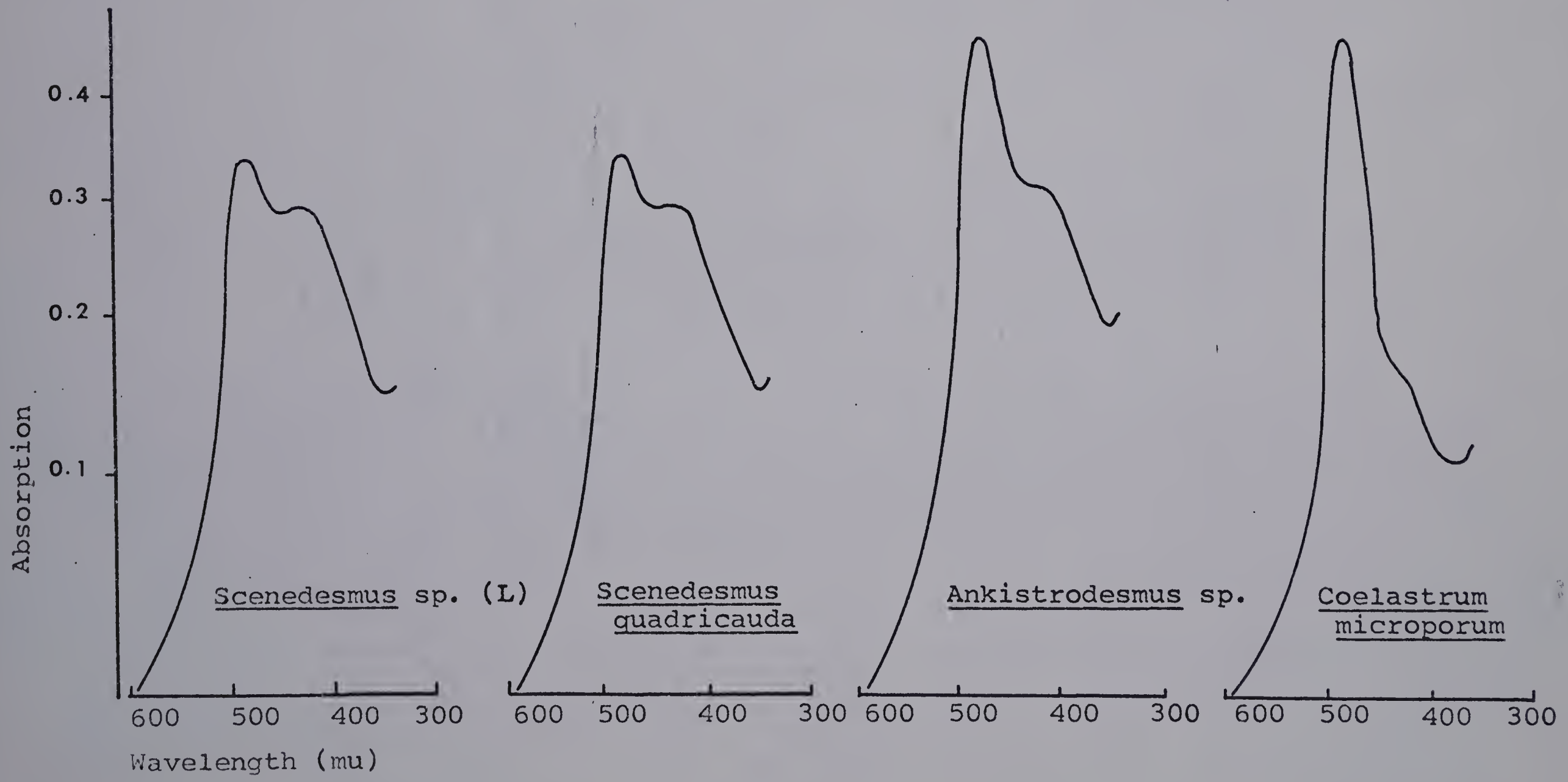


Fig. 8. Absorption spectra of Fraction C extracted from particulate matter in Lower Pond at different time.

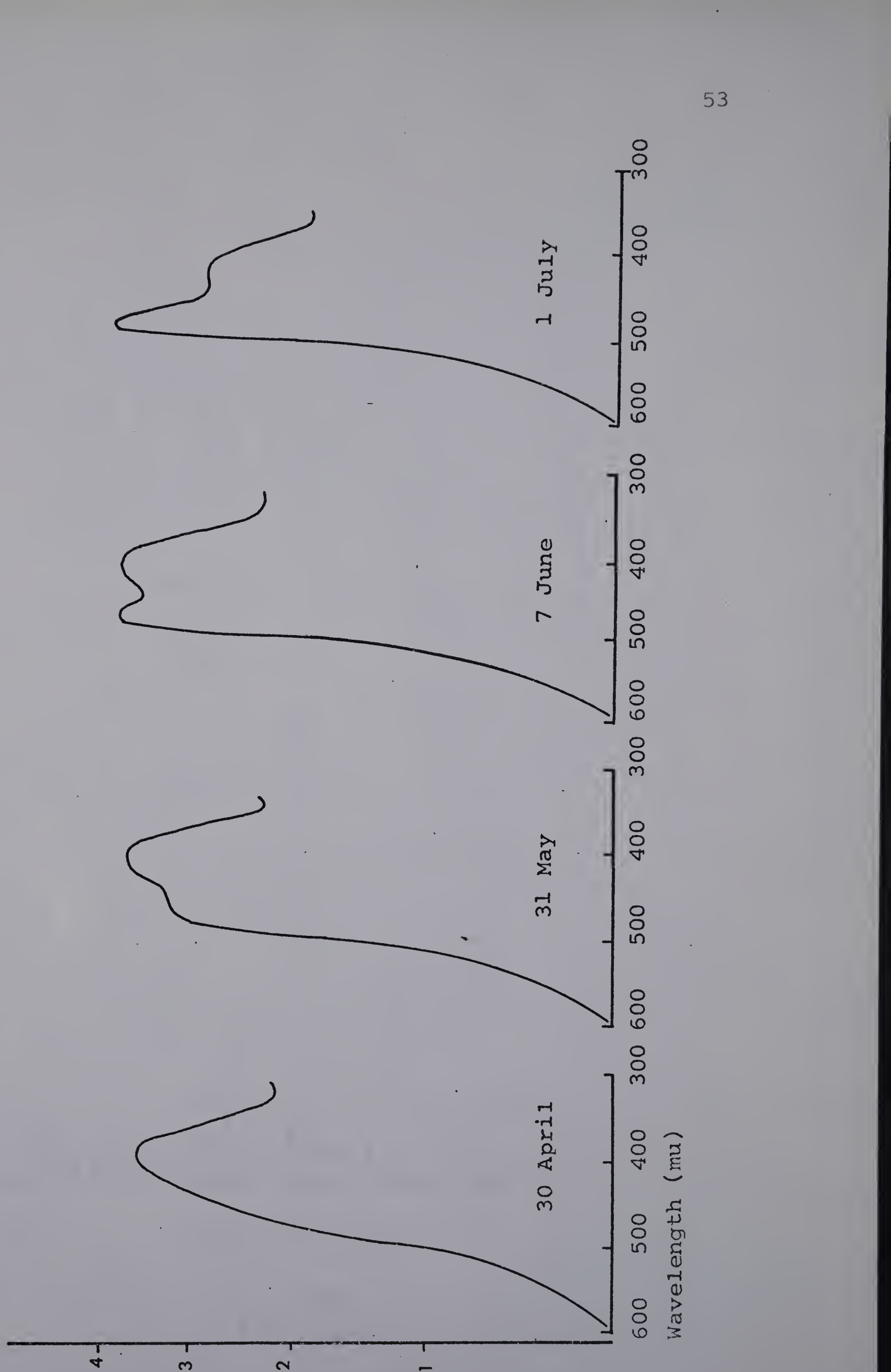
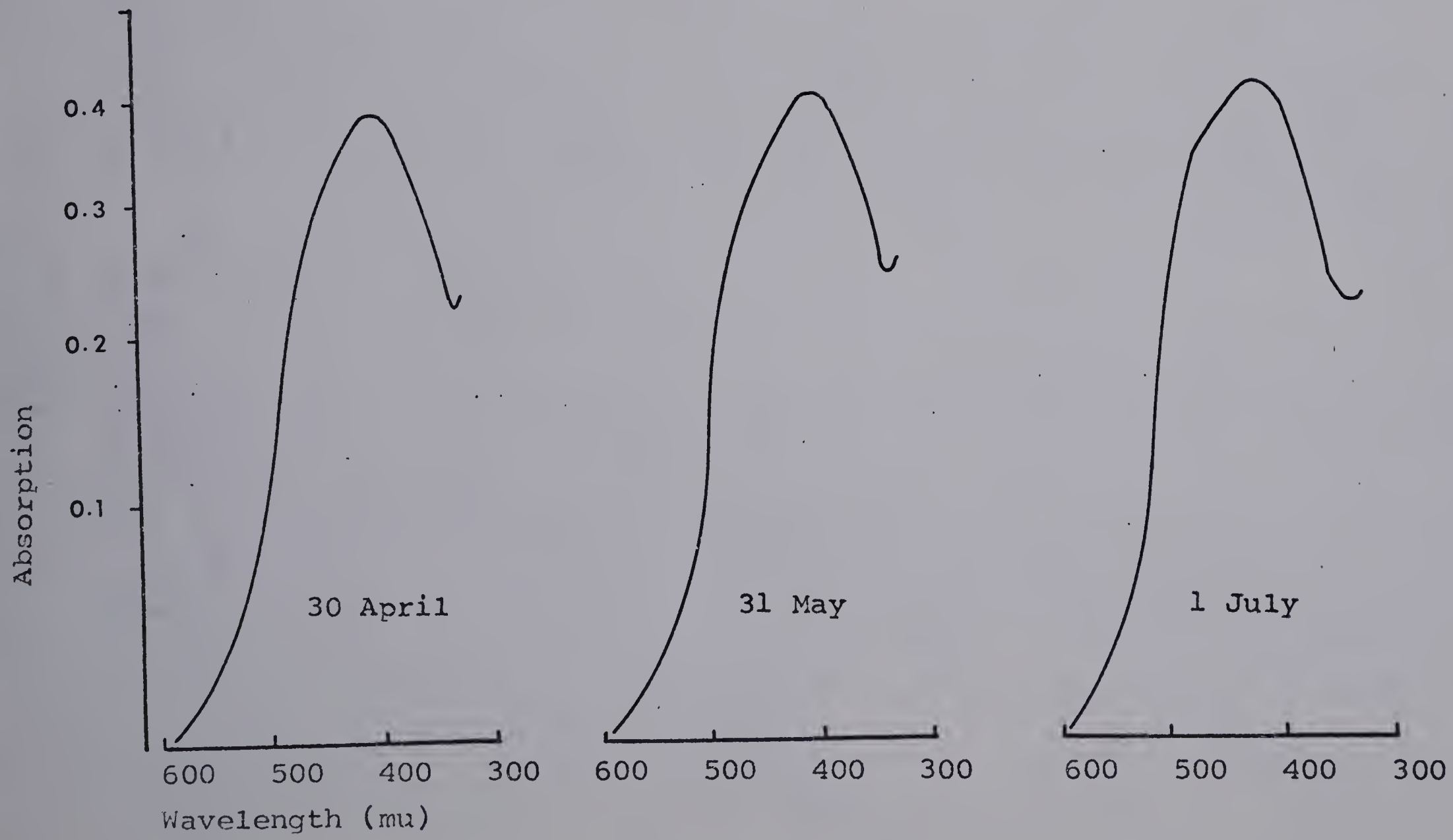


Fig. 9. Absorption spectra of Fraction C extracted from particulate matter in Upper Pond at different time.



populations reached a high level. When the maximum population was reached, the absorption spectra of particulate matter were very similar to those of the algal cells. These observations indicated that: (1) cellulose fractions were closely associated with the chlorophyll complex in particulate matter and (2) algae were the major cellulose contributors in this system.

Since the absorption maxima of the second peaks were different from those of glucose and related compounds, there was no reason to suspect that the presence of the pigments interfered with the reducing sugar determinations of the algal cells. However, in those situation where small algal crops were observed, the presence of the pigment complex could have masked the diagnostic peak used to determine amounts of reducing sugars in particulate matter and give values for C-fractions that were higher than they actually were (Figs. 8, 9).

Cellulose Production of the Phytoplankton to the Water Column

Cellulose actually contributed by the observed phytoplankton crops was estimated by multipling the approximate cellulose value per cell by the number of cells of each type observed. In the case of green algae, estimates of cellulose production were based on cellulose values obtained in this study (Table 4-6). Cellulose values per cell for dinoflagellates, Ceratium and Peridinium (Table 9), were extrapolated from dry weights and volumes cited in the literature (Lund, 1959; Mullin et al., 1966). The values obtained are submitted as rough approximation. These data make the obvious point that even

Table 9. Estimated cellulose composition of other cellulose producing algae (dinoflagellates).

Species	Dry wt. [a] ($ug/10^6$ cell)	Estimated cellulose [b] ($ug/10^6$ cell)
Ceratium hirundinella	20,000	2,898
Peridinium cinctum	35,000	5,071

[a] The dry weight of Ceratium and Peridinium were taken from Lund (1962).

[b] The dry weight of Ceratium was 10 times greater than Staurastrum orbiculare; since the cellulose content of Staurastrum was estimated experimentally, this value was used for estimation of cellulose content.

The estimated cellulose value of Peridinium was also based on the dry weight of Staurastrum.

Table 10. Comparison of observed and calculated values of cellulose in water column.

Sampling month	Observed in particulate matter (PM)		Calculated from phytoplankton population		% dry wt from phyto-plankton	
	Dry wt of PM (mg/L)	Cellulose (ug/L)	Dry wt of PM (ug/L)	Cellulose (ug/L)	PM	Cellulose
Lower Pond						
Jan	2.4	362	9.0	1.8[a,b]	0.4	0.5
Feb	2.4	307	3.9	1.0[a,b]	0.2	0.3
Mar	2.1	230	2.8	0.3[a,b]	0.1	0.1
Apr	1.5	250	17.0	4.7[a,b]	1.1	1.9
May	2.6	373	110.0	21.0[a,b,d]	4.2	5.6
Jun	5.6	560	277.3	45.9[a,d]	5.0	8.2
Jul	5.2	553	1,012.2	169.1[a,d]	19.4	30.6
Upper Pond						
Jan	2.8	450	0.1	NG	NG	NG
Feb	2.3	370	0.3	NG	NG	NG
Mar	2.3	317	0.3	0.1[c]	0.01	0.03
Apr	1.1	250	5.0	0.7[a]	0.5	0.2
May	2.0	178	1.7	1.3[a]	0.1	0.7
Jun	3.1	248	8.0	1.1[a,b]	0.3	0.4
Jul	4.4	196	268.2	16.0	6.1	8.2
Station D						
Feb						
1 m	1.4	192	36.4	5.3[a]	2.6	2.8
8 m	1.2	180	50.7	7.2[a]	4.2	4.0
Jun						
1 m	1.2	132	5.0	0.6[d]	0.4	0.4
8 m	1.5	144	35.0	6.0	2.3	4.1

The calculated values did not include the C-fraction of *Dinobryon* (a), *Gymnodinium* (b), *Closterium* (c) and *Golenkinia* (d).

though a significant population of cellulose-producing algae was present in the water column, there was more cellulose observed in the particulate matter than could be accounted for by the algae. That this kind of observation was quite common is shown in Table 10.

Vertical Distribution of Cellulose and Related Carbohydrates

The distribution of cellulosic material in the water column was observed at Station D and H during the summer stratification period in 1969. This was done because it was desired to determine the fate of cellulose for the two systems in which the origins of the cellulose differed. Table 11 showed the distribution of cellulose and related carbohydrates with respect to temperature, particulate matter and phytoplankton. The amounts of cellulosic materials did not appear to change with depth, while the amounts of related carbohydrates decreased in the hypolimnion at Station D; an area presumed to contain little particulate matter of exogenous origin. However, both cellulose and related carbohydrate fractions were increased significantly in the hypolimnion at Station H; an area presumed to contain considerable particulate matter of exogenous origin.

A comparison of the cellulose and related carbohydrates with phytoplankton crops suggests that a sinificant fraction of detritus was present in the hypolimnion. Carbohydrates, other than cellulose, were degraded at a rapid rate above the thermocline. This could have accounted for the concurrent decrease in the Fraction S+H/C ratio with depth. A typical set

Table 11. Distribution of selected parameters during summer stratification.

Station D

Depth (m)	Temp. (C)	Particulate matter (PM) (mg/L)	Carbohydrate fraction (ug/L)				Cellulose-containing phytoplankton (units/L)
			S	H	C	S+H/C	C as % dry wt.(PM)
10 July							
1	22.5	0.63	66.8	38.1	205.0	0.51	32.5	10,900
5	22.3	0.90	93.0	30.0	200.0	0.61	22.2	27,000
9	20.5	1.20	96.7	31.5	264.7	0.48	22.0	26,400
14	14.0	0.57	55.7	19.0	280.0	0.27	49.1	7,300
17	13.5	0.53	66.7	17.5	243.0	0.35	45.8	4,700
22 July							
1	23.5	0.38	39.5	33.8	167.5	0.44	44.1	69,000
5	23.5	0.43	38.8	37.5	154.0	0.49	35.8	160,900
9	21.7	0.63	41.3	33.8	168.0	0.45	26.7	117,800
14	15.2	0.48	36.8	36.4	158.0	0.46	32.9	34,300
17	13.7	0.33	24.3	18.0	145.0	0.29	43.9	24,600

Table 11. continued

Station H

Depth (m)	Temp. (C)	Particulate matter (PM) (mg/L)	Carbohydrate fraction (ug/L)					Cellulose-containing phytoplankton (units/L)
			S	H	C	S+H/C	C as % dry wt.(PM)	
10 July								
1	24.9	1.65	76.5	30.0	153.0	0.69	9.3	41,000
4	24.7	1.33	86.0	40.0	223.3	0.56	16.8	21,400
8	19.0	1.12	90.0	27.0	224.0	0.50	20.0	8,800
12	14.7	2.70	90.0	24.0	420.0	0.27	15.5	6,800 (debris)
16	13.4	22.71	407.1	96.4	1,185.0	0.42	5.2	debris
31 July								
1	23.5	0.70	44.5	61.1	174.5	0.60	24.9	258,000
4	23.4	0.55	43.8	54.8	170.0	0.58	30.8	316,500
8	21.8	0.65	38.8	30.8	170.5	0.40	26.2	96,000
12	19.0	3.15	96.5	61.5	502.0	0.31	15.8	1,300
16	--	15.65	177.5	180.0	557.0	0.64	3.5	600 (debris)

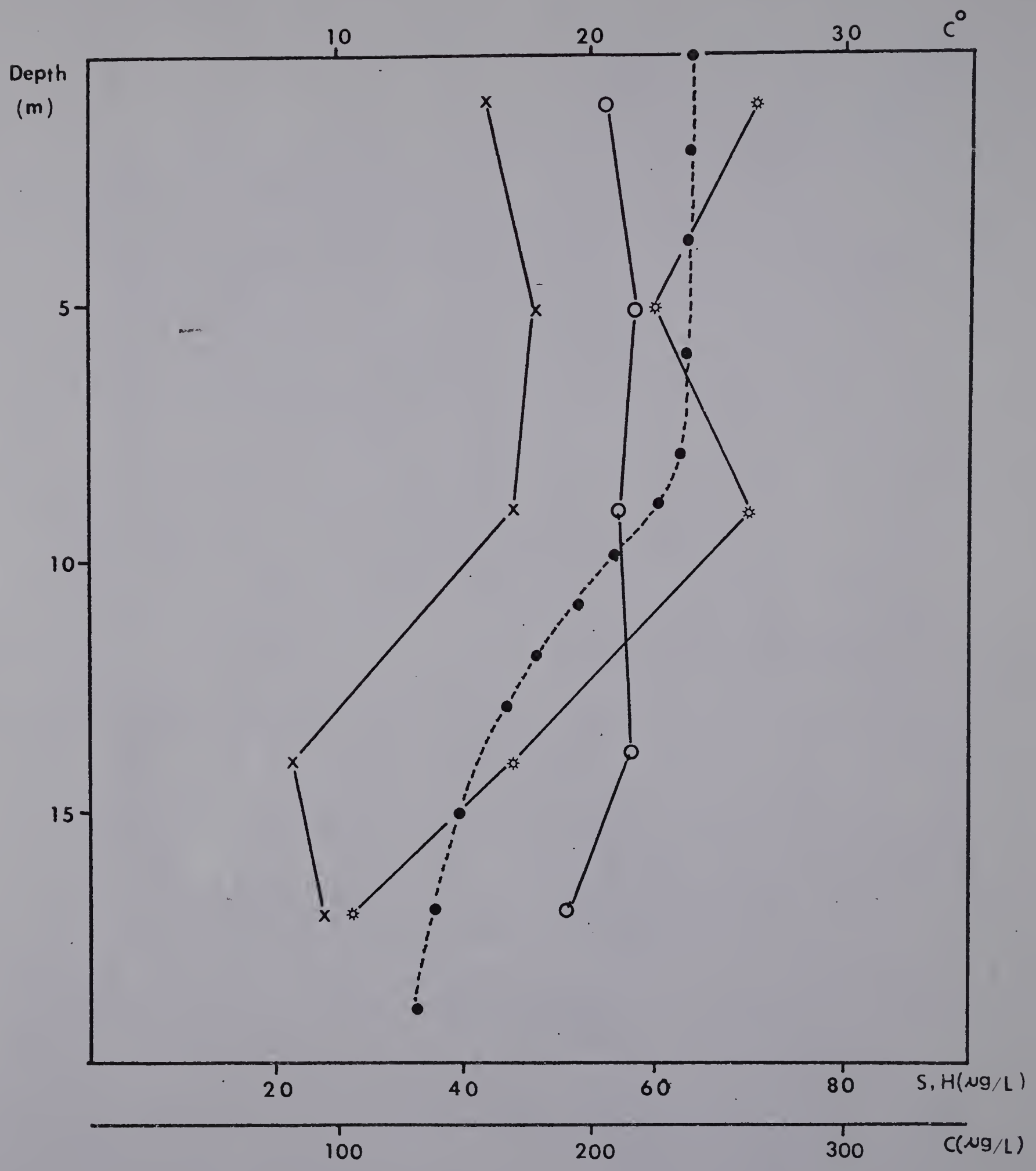


Fig. 10. Vertical distribution of Fraction S (✲—✲), H (x—x), and C (o—o) with respect to temperature profiles (•---•) at Station D (August 7, 1969).

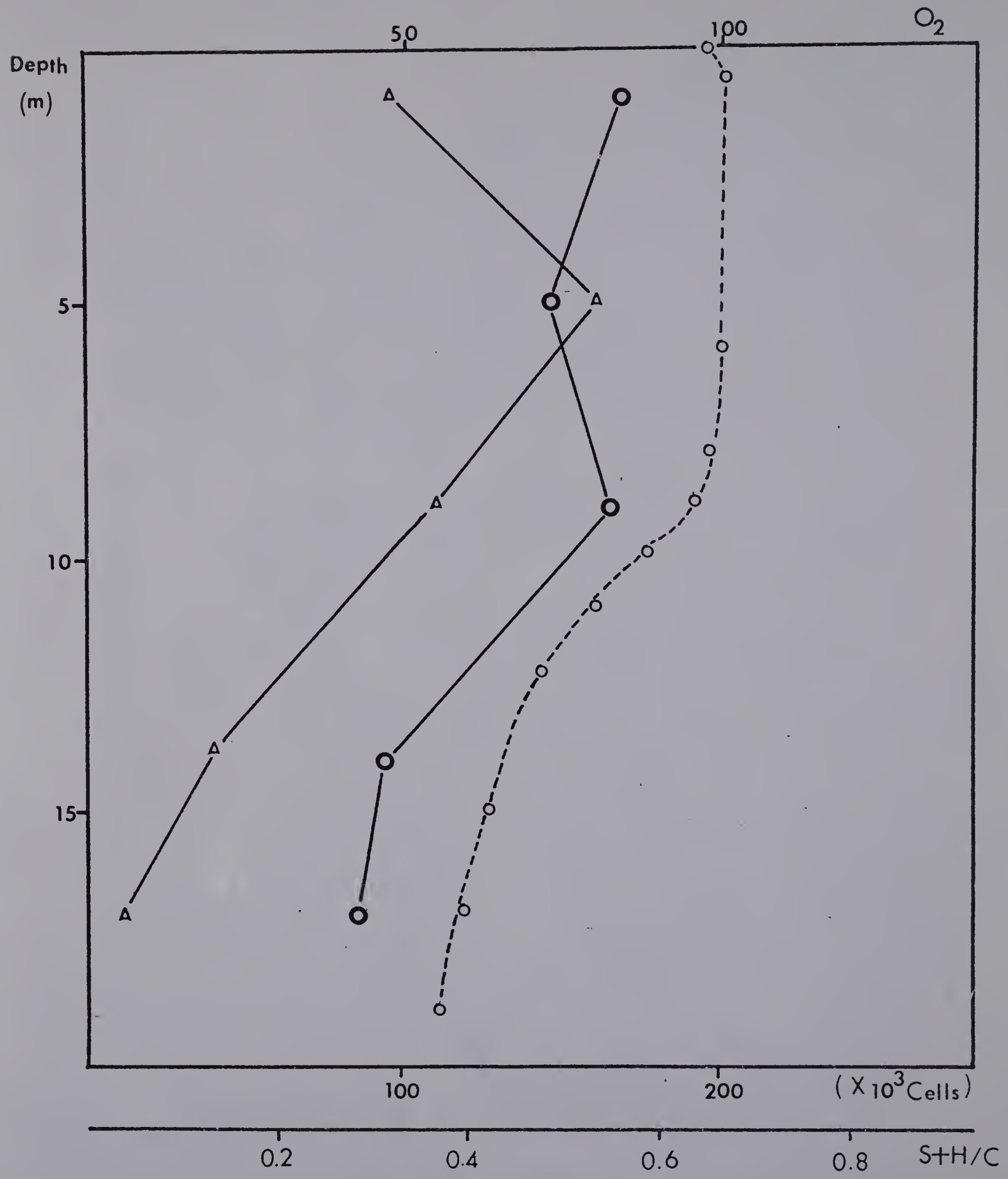


Fig. 11. Vertical distribution of S+H/C ratio (O—O) with respect to phytoplankton crop (Δ—Δ) and dissolved oxygen profiles (o···o) at Station D (August 7, 1969).

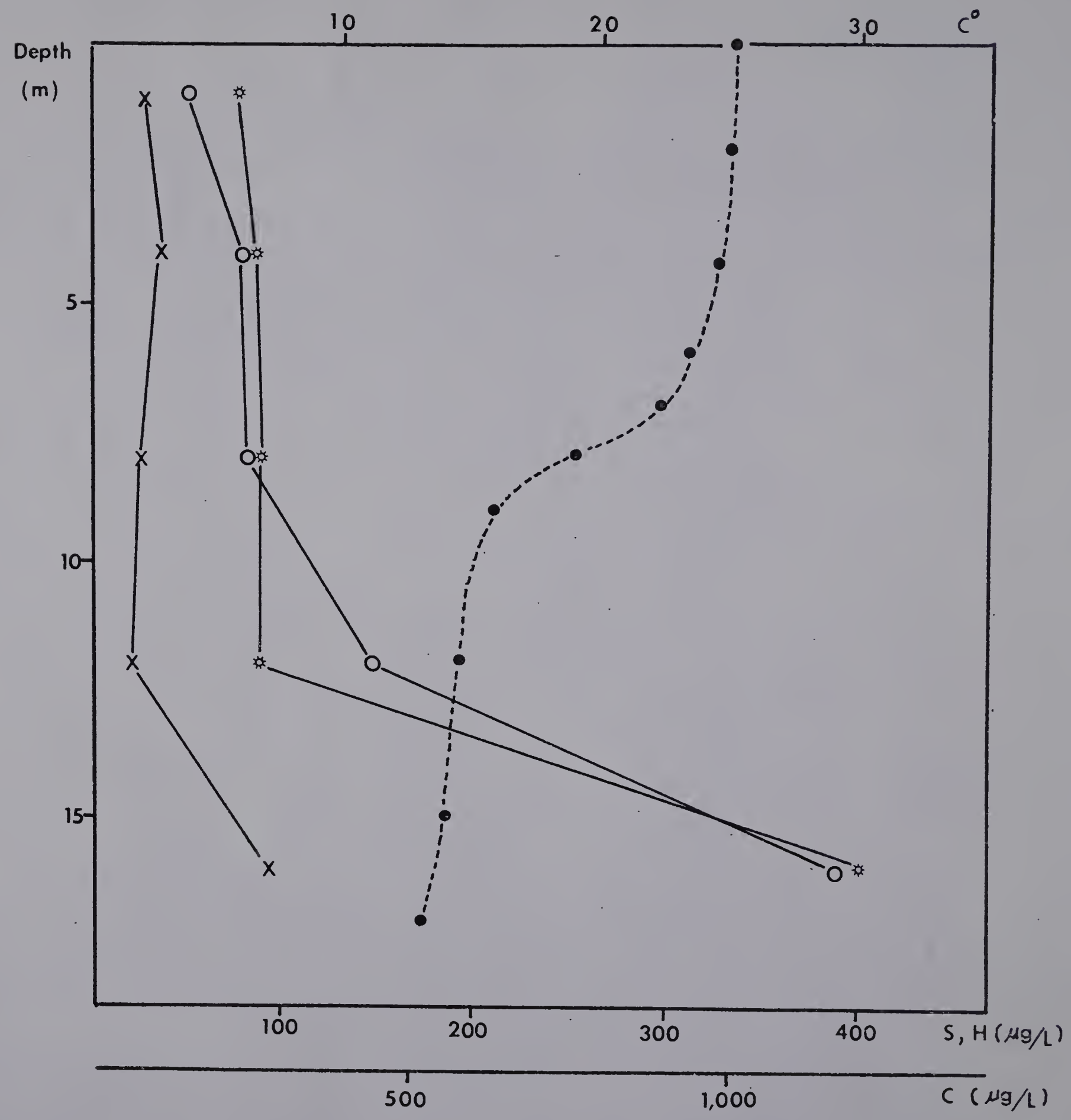


Fig. 12. Vertical distribution of Fraction S (✡—✡), H (X—X), and C (O—O) with respect to temperature profiles (●···●) at Station H (July 17, 1969).

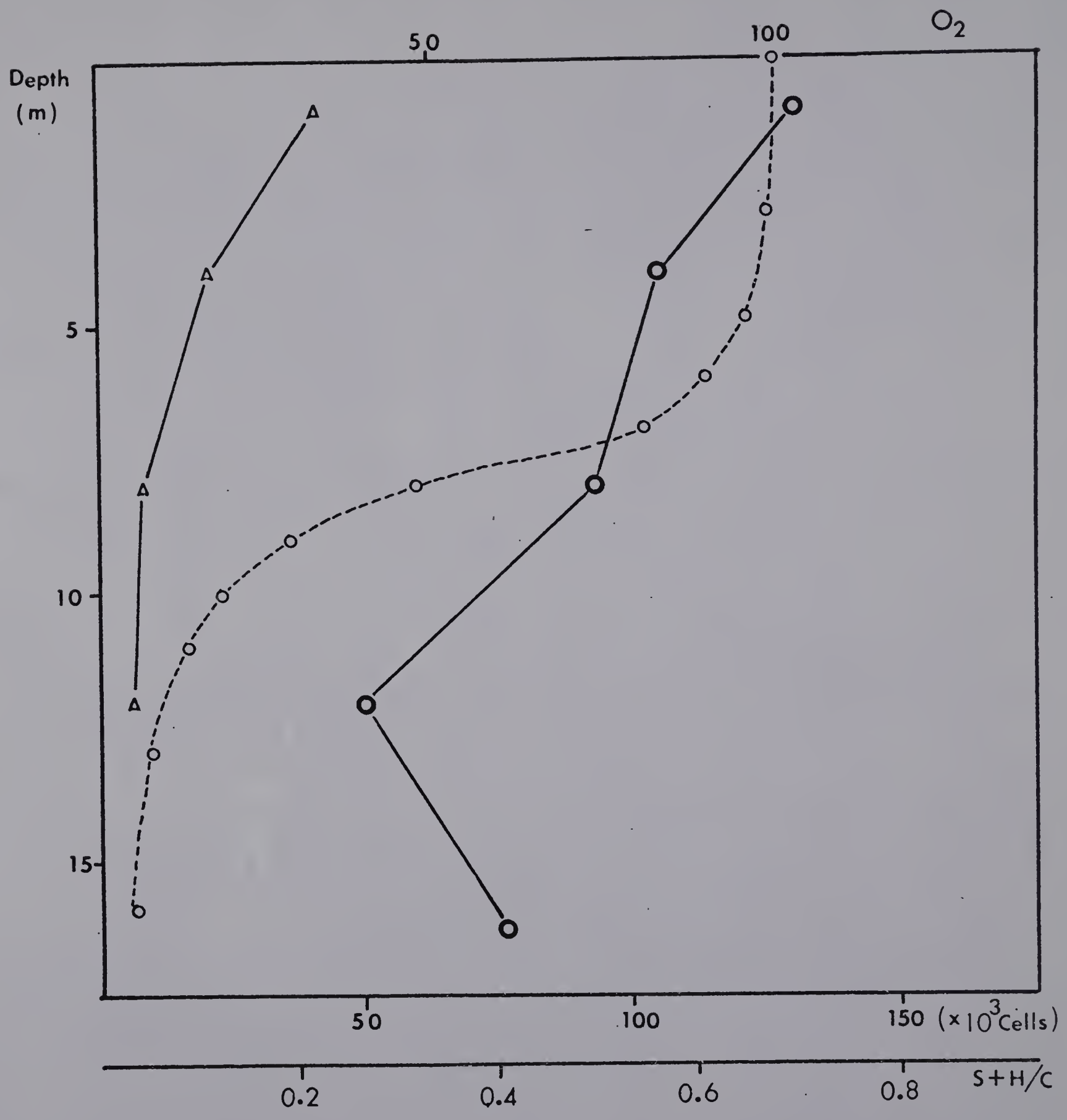


Fig. 13. Vertical distribution of S+H/C ratio (O—O) with respect to phytoplankton crop (Δ—Δ) and dissolved oxygen profiles(o---o) at Station H (July 17, 1969).

of observations at Station D and H is illustrated in Figs. 10-13. This was quite common during stagnation period (Appendix II, Table 2,3).

Such observations imply that the non-cellulosic carbohydrates were degraded at a more rapid rate than cellulosic material in the upper portion of the water column. The ultimate fate of cellulosic material appeared to be high resistance to decay and settling to the bottom.

DISCUSSION

Phytoplankton Composition

It is generally accepted that a complete understanding of the productivity of a water mass requires detailed knowledge of the composition of the phytoplankton population present. Similarly if one wishes to understand the details of cellulose production in an aquatic environment, information pertaining to the numbers and types of cellulose containing planktonic algae is essential.

As has been the case in other studies, the difficulties in making determinations of numbers, types, sizes and distribution of phytoplankton in the natural system were several: the algal cells in the water column were not distributed in a uniform manner; many types were present in very low concentrations requiring the concentration of relatively large-volume samples; the concentration techniques available tended to break up the colonial forms and cells of delicate species, and to concentrate detritus which made microscopic examination difficult.

The data presented in Table 1,2 and 3 show that relatively few types of algae were observed. However, since these studies were undertaken with the intention of ascertaining the dominant species of cellulose-producing phytoplankton, it was deemed reasonable to restrict the scope of inquiry, omitting those species which were not known to be cellulose producing, or which were present in low numbers. This restriction allowed greater opportunity for concentration on dominant organisms, and

therefore, attention was directed to fewer species than has been evidenced in some other reports.

The composition of phytoplankton during the periods of investigation demonstrated that, of the cellulose-containing algae, the members of Chlorophyceae were the most abundant in all areas studied. Other significant cellulose-producers, members of Dinophyceae and Chrysophyceae, were present, but never abundant. Members of these three groups accounted for more than 70% of total phytoplankton observed in the productive Lower Pond. Similar results were observed in the Upper Pond and Quabbin Reservoir. These figures compare favorably with the findings of Hilliard (1965) for an Alaskan pond where desmids (42.2%), Chlorococcales (25.7%) and Chrysophyceae (21.6%) were reported. The composition of phytoplankton in temperate lakes of eutrophic and oligotrophic nature in Europe indicated that chlorophyceans were abundant during summer months (Skulberg, 1964). However, the above figures are contrary to the findings of Spencer (1950) for Quabbin Reservoir; Tryon and Jackson (1952) for Pymatuning Lake, Pa.; and Griffith (1955) for Lake Michigan. In these earlier studies, it was found that less than 10% of the total phytoplankton belonged to Chlorophyceae and Dinophyceae.

It should be noted that green coccoid algae, the majority of which are constituted by the genera Chlorella and Oocystis, were dominant in each of the natural systems I investigated. This is contrary to the observations of Edelstein (1965), who stated that the green algae never become the dominant flora

despite the fact that a preponderance of the species found in lacustrine environment are members of this group. The difference between my observations and those of Edelstein may be explained by the chemical nature of water. Reasonable conclusions concerning abundance of the Chlorophyceae are difficult, however, since green algae occurred in times of low pH (Spencer, 1950), the dominance of this group in the systems I have studied may be the result of the slightly acidic reactions of each of these bodies of water. It is apparent that the composition and periodicity of phytoplankton are influenced by many factors, and that many genera have environmentally-specific requirements. In this case, chemical factors such as the pH level were highly important in determining the species-composition of the lake.

While it has become something of an accepted limnological fact that the composition of phytoplankton is correlated with the trophic stage of the lake, the results of this study do not tend to support that notion. My results show that species of Dinobryon and desmids, often accepted indicators of oligotrophy, were abundant in both the productive Lower Pond and the unproductive Quabbin Reservoir. Further, blue-greens, often characteristic of members of phytoplankton in eutrophic systems, were numerous in Quabbin Reservoir. Perhaps such apparent anomalies may be explained by the hypothesis that selected essential nutrients are more significant determinants of the algal types present than the over-all productivity of the systems (Sparling and Nalewajko, 1970).

Cellulose Contents of Planktonic Algae

An abundance of cellulose containing algae, members of Chlorophyceae, Dinophyceae and Chrysophyceae, were observed in the water column. Therefore, an attempt was made to isolate and culture these algae in order to estimate their cellulose contents. Of those isolated, fourteen representative algal species were selected and analyzed for cellulose, and the results show that dry weight values are similar to those obtained by other investigators (Lund, 1962; Nalewajko, 1966). The percent cellulose values for Chlorella sp. (1.5-4.2%) were similar to the recalculated value (2.1%) reported by Northcote et al. (1958) for a similar species. The former values represent the percent cellulose content of cells taken from the exponential growth phase (1.5%) and from the stationary growth phase (4.2%). However, Northcote did not indicate the growth phase of reported cells, and an extensive review of the literature yielded no other information regarding cellulose content of other species.

Lund (1959) reported that the dry weight of desmids was about 500 to 2,500 times that of Chlorella, while the dry weight of Peridinium, on the other hand, was approximately 9,000 times that of Chlorella. It is no wonder, then, that dry weight estimates of crops of the larger forms were usually higher than those of smaller algae. Variations in the cellulose contents of the cells were similar to those of the dry weights of such algae. It was found that larger algal cells contained much greater amounts of cellulosic constituents per cell (Table 6) than the smaller cells (Table 4). The cellulose content of

large desmids was about 1,700-10,000 times that of Chlorella. Clearly, the larger algae have long generation times which will permit greater synthesis of cellulosic material; conversely, the smaller algae have short generation times and, therefore, produce less cellulose per cell. However, while smaller algae produce less cellulose, they were more numerous than the larger forms in the system studied. Seen on this level, the rapidly multiplying smaller species may produce more cellulose in a given period than the larger algae. Thus smaller algae must be considered important cellulose producers.

The results of this investigation, which employs a limited number of algal species, have shown that 2-39% of the total dry weight of the algal crop was cellulosic in nature. Since algae are often the main source of detritus in lakes (Rodina, 1963; 1966) and since the cellulose fraction appears to be a major part of algal crops, cellulose fractions in particulate matter probably reflect the relative abundance of past and present phytoplankton crops. Thus, it appears that the numbers and types of algae present in the water column could be primary factors regulating the amounts of cellulose present in such systems.

Cellulose Content of Water Column

This study demonstrated that 4-50% of the total dry weight of the particulate matter present in all systems studied was cellulose (Appendix II, Table 2,3). In this short term study, no direct relationships between the amount of cellulose in the system, and phytoplankton populations, total amount of particu-

late matter, total oxidizable carbon, or trophic stages, were evident. In general the cellulose content was observed to be higher in the eutrophic and dystrophic system than in the oligotrophic system. Presumably, this was due to the fact that larger phytoplankton crops are more probable in eutrophic systems and dystrophic systems than in oligotrophic systems.

The data in Table 8 shows that concentrations of oxidizable carbon in the particulate matter were high where phytoplankton populations were high and that concentrations of cellulose were independent of phytoplankton production; i.e., an increase in the amounts of oxidizable carbon was not necessarily accompanied by an increase in the amounts of cellulose observed. In addition, the percentages of the particulate matter estimated as cellulose in Quabbin Reservoir and Upper Pond were higher during winter months than summer months, while the reverse situation was found in the productive Lower Pond. Several factors may be involved in the explanation of these apparent relationships: (1) summer and winter sampling conditions were different in shallow water station because bottom sediments that may have been relatively rich in cellulose were probably disturbed by ice removal during winter sampling processes; (2) falsely higher spectrophotometric measurements of glucose could have been obtained during the winter months since a pigment complex peak could have masked the diagnostic glucose peak; (3) zooplankters were observed in the samples taken from Upper Pond and Quabbin Reservoir during the summer months and the predation of phytoplankton by zooplankton might be an important factor, (4) the previous intact

cellulose fraction might be degraded when the optimal conditions for microbial digestion associated with the summer months occur.

Short term observation of the cellulose content in the three systems showed that this material varied 2 orders of magnitude during the period of investigation while the oxidizable carbon contents varied 8 orders of magnitude. This suggests that the amount of cellulose produced in the water column accounts for a relatively small proportion of the particulate organic matter produced by algal populations that can be recycled rapidly. The abundance of cellulosic material in the water column may be explained by its selective resistance to decomposition.

It was assumed that the primary source of cellulose in the Lower Pond, Upper Pond and Station D was of algal origin because more than 70% of total phytoplankton encountered in the water column were cellulose producing algae. In the case of Station H, it can be assumed that much of the cellulose of particulate matter were of exogenous origin. Further, 2-39% of total dry weight of algal cells was of cellulosic material. Interpretation of spectrophotometric determinations of the particulate matter gave additional support to this assumption. The C-fractions extracted from algal cells, as well as those from particulate matter yielded spectra which differed slightly (in the range of 400-450 mu) from both those obtained with hydrolysates of purified cellulose, and those taken from higher plants. Apparently, the C-fractions from algae contained a pigment complex not found in the C-fractions from higher plants and from purified cellulose. Thus, the similarity between the obser-

vation spectra of C-fraction from algae and those of particulate matter indicate that a significant portion of the cellulose in particulate matter could have been of algal origin.

This conclusion, that the cellulose fractions present in particulate matter are associated with phytoplankton crops, is supported from other quarters. In addition to the above, seasonal variations of the absorption pattern of C-fraction from the Lower Pond (Fig. 8) demonstrated that when large populations are present, the absorption patterns are similar to those of algal cells. When small algal crops were observed, the diagnostic peak (associated with glucose) appeared to be masked by a second peak (associated with pigment complex). The evidence presented in Figs. 6-7 suggests that the second peak appears in C-fractions of algal cells, and not in those of higher plants; i.e., this appears to be associated with algal materials. Accordingly, most or all of the cellulose in the particulate matter in the systems studied, with exception of Station H, appeared to be of algal origin. This tends to be consistent with the microscopic observation made by Rodina (1963), who found the detritus in the water column was composed of dying algae, and the amount of the detritus of higher aquatic vegetation was insignificant.

Estimation of Cellulose Contribution by Phytoplankton Population

Since significant numbers of cellulose-producing algae were present in the water column, and a considerable portion of cellulosic material in the particulate matter may have been of algal origin, an attempt was made to estimate the amounts of

cellulose actually contributed to the water column by the phytoplankton present. Efforts were made to determine the cellulose content per cell of the important algal types encountered in the water column. However, the results of these efforts are incomplete due to the difficulties cited earlier.

It was practicable to determine the cellulose content of Ceratium, Peridinium and Dinobryon, which may be high cellulose producers. However, values for Ceratium and Peridinium could be extrapolated from dry weights cited in the literature (Table 9). No information on Dinobryon was found which could be used in the computation of cellulose production by this alga.

The extrapolated values obtained for Ceratium and Peridinium must be considered as crude approximations. Further, since photosynthetic activity of cells is influenced by such factors as temperature, exposed light intensity, nutrients and cell ages, there was good reason to suspect that the cells studied in the laboratory differ greatly from those in the natural enviornment.

Nevertheless, an estimation of the amounts of cellulose actually contributed to the water column by phytoplankton populations was attempted by multipling the approximate cellulose values per cell by the number of each of the types observed. If the C-fractions measured were taken primarily from intact algal cells, it was assumed that the measured C-fractions and the estimated C-fractions would correlate. The data in Table 10 show that when algal populations in the productive Lower Pond reached a maximum during the summer months, 8-30% of the cellulose fraction present in the particulate matter could be accounted for

by the phytoplankton. The low percentage of cellulose (0.1-2%) in this pond during winter and spring months that could be accounted for by phytoplankton crops present was due to the low population of phytoplankton as well as to the accumulation of detritus. In unproductive systems, as might be expected, the lower values for cellulose fractions were accounted for by low populations of phytoplankton. Similar patterns were obtained in the production of particulate matter by phytoplankton populations.

It is clear that there was more cellulose present in the water column than could be accounted for by the number of algal cells observed. This suggests that the cellulose produced by algal cells is not decomposed at a significant rate, but remains in the water column for a long period of time. It is possible that this refractory material could become a part of more complex macromolecules.

The Fate of Cellulose in the Water Column

The relative abundance of carbohydrate fractions S, H and C in the particulate matter differed from those derived from algal cells. C-fraction concentrations were higher than S and H-fractions in particulate matter (Table 8), while the results for algal cells were the reverse (Table 4). Such abundance of C-fraction in particulate matter could be due to the accumulation of previous algal cell residues, which suggests that C-fraction of algae is resistant to further decomposition in the water column. If the carbohydrate fractions were completely decomposed and went into solution or settled out after algal blooms, there

would be no accumulation of detritus. Although this is possible in a hypothetical system, the decomposition of organic matter is rarely complete in nature.

Residues from dead algal cells together with living cells are then always present in the water column. However, the settling rate of dead cells is several times greater than that of living cells (Eppley et al., 1967), and this faster settling of dead cells resulted in a decrease in the phytoplankton crop, but an increase of detritus with depth. The data of the vertical distribution of Fraction S+H/C ratio can be explained in terms of the decomposition balance of the carbohydrate fractions in the water column. Figs. 10-13 show that the vertical distribution of the Fraction S+H/C ratio is related to that of the phytoplankton crop, as well as to the temperature and oxygen profiles. The decrease in the S+H/C ratio with depth indicates that the disappearance of Fraction S+H occurs more rapidly than that of Fraction C. The rapid decrease in S+H/C ratio below the thermocline indicated that Fraction S+H decomposed very quickly as the dead phytoplankton sank to the bottom. For this reason, Fraction C may be a major component of particulate polysaccharides on the bottom of a lake. These observations basically agree with those of Kleerekoper (1953), who observed that most decomposition of sinking detritus takes place in the epilimnion of the lake. A similar observation was reported by Handa (1970), who found that water-insoluble carbohydrates remain intact at a depth of 1,000 m. It, therefore, seems highly probable that a considerable part of the particulate

carbohydrate fractions at the bottom may be considered a net loss in the amount of nutrients present.

There is little doubt that bacterial action brings about the decomposition of planktonic remains in lakes. Previous studies report that cellulolytic bacteria were present in water columns (Reynolds et al., 1968) and very large numbers of Cytophaga and Sporocytophaga were found in detritus (Rodina, 1963). However, as indicated earlier, the decomposition of cellulosic material may be incomplete in some natural situation. If significant cellulolysis occurred, there should have been little or no accumulation of cellulose in the water column. The results of this study have indicated that there were accumulations of such material in the water column and that they appeared to increae with depth.

Two main conclusions can be drawn from a careful comparsion of the results of this study with those of previous efforts. First, the role of cellulose-producing algae in the origin of much of the cellulose fraction in the water column emerges when a global account is given of the evidence from these three factors: the incidence of cellulose-producers; cellulose contents of representative algae and particulate matter; and absorption spectra of C-fraction from various sources. Despite its algal origin, there was always more cellulose present in the water column than could be accounted for by the algal crop observed. This may be due to the fact that the cellulose from previous algal populations was not being removed.

Secondly, although previous studies reported the presence

of cellulolytic bacteria in the water column, there is no evidence of the activity of such bacteria, and I have found little to suggest that C-fraction degradation occurred there. The vertical distribution of the phytoplankton crops and carbohydrate fraction S+H/C ratio in stratified lakes, indicated that carbohydrate fractions S and H were degraded easily enough- as dying algal cells settled- but the C-fractions remained intact and in fact accumulated with depth.

It has been supposed that this material is mineralized in bottom sediments (Kleerekoper, 1953; Whittaker and Vallentyne, 1957) and serves as a constant source of organic matter (Alexander, 1965). However, it is premature to speculate on the fate of such material on the bottom because descriptive data is lacking for the microbiological and biochemical events at the mud-water interface. It may be that the cellulolytic bacteria mentioned in the earlier reports are inactive in the water column but degrade cellulosic materials at the mud-water interface.

SUMMARY

1. Estimations were made of the numbers and types of cellulose containing algae in three bodies of water; an eutrophic pond (Lower Pond), a dystrophic pond (Upper Pond) and an oligotrophic lake (Quabbin Reservoir). These algae were estimated to be approximately 70% of total phytoplankton population observed.
2. The cellulose content of fourteen algal species representing the dominant cellulose containing phytoplankton varied from 2 to 39% of the dry weight of the cells studied. The cellulose content was dependent upon the species of algae studied and their stages of growth. Although the larger algal cells contained greater amounts of cellulose per cell than the smaller algae, the smaller algae were more numerous, had shorter generation times, and were considered to be the important cellulose producers.
3. The amounts of cellulose present in the water column ranged 110 to 1,185 ug/L, which accounted for 4-50% of the total dry weight of particulate matter.
4. A comparison of absorption spectra of the extracts from algae, higher plants, particulate matter from bodies of water studied and purified cellulose, indicated that the cellulosic material present in particulate matter was of algal origin.
5. It was estimated that 0.1-30% of the cellulose in the water column was actually contributed by phytoplankton crops observed at the time of sampling. The low value (0.1%) was

observed when the phytoplankton populations were low, and the high value (30%) was obtained when the highest algal population was observed. Because there was more cellulose found in the water column than could be accounted for by the number of algal cells observed, this difference may be due to the accumulation of cellulose from previous algal crops.

6. The fate of cellulosic material was determined by comparing the vertical distribution of different carbohydrate fractions (soluble carbohydrate, S; hemicellulose, H; cellulose, C) in the water column. The decrease in the S+H/C ratio with depth indicated that fractions S and H were degraded in the water column, while fraction C remained intact and settled to the bottom.

7. It may be concluded that the cellulose fractions in the water of three areas studied were produced by phytoplankton. These fractions were not decomposed and settled to the bottom. Thus, it appeared that little or no decomposition of cellulosic material took place in the water column.

APPENDIX I

Table 1. Phytoplankton of oligotrophic and eutrophic lakes.

Rawson (1956)

	Oligotrophic	Eutrophic
Quantity	poor	rich
Variety	many species	few species
Water-blooms	very rare	frequent
Characteristic algal groups and genera	Chlorophyceae	Cyanophyceae
	Staurastrum	Anabaena Microcystis Aphanizomenon
	diatoms	diatoms
	Tabellaria Cyclotella	Melosira Fragilaria Asterionella Stephanodiscus
	Chrysophyceae	
	Dinobryon	
Quotient		
simple:	number of species of $\frac{\text{Chlorococcales}}{\text{Desmidiaceae}}$ =	if <1, oligotrophy if >1, eutrophy
compound:	$\frac{\text{Cyanophyceae+Chlorococcales+Centrales+Eugleniaceae}}{\text{Desmidiaceae}}$ =	if <1, oligotrophy if >2.5, eutrophy

APPENDIX II

Table 1. Polysaccharide fractions recovered from commercially purified cellulose.

Source	Carbohydrate fraction (% dry wt)			Total carbohydrate (% dry wt)
	S	H	C	
Solka-floc (SW-40-A)	1.2	1.0	87.8	90.0
DP-1000	0.1	0.2	98.5	98.8

Table 2. Distribution of selected parameters during summer stratification.

Station D

Depth (m)	Temp. (C)	Particulate matter (mg/L)	Carbohydrate fraction (ug/L)					Cellulose-containing phytoplankton (units/L)
			S	H	C	S+H/C	C as % dry wt	
10 July								
1	22.5	0.63	66.8	38.1	205.0	0.51	32.5	10,900
5	22.3	0.90	93.0	30.0	200.0	0.61	22.2	27,000
9	20.5	1.20	96.7	31.5	264.7	0.48	22.0	26,400
14	14.0	0.57	55.7	19.0	280.0	0.27	49.1	7,300
17	13.5	0.53	66.7	17.5	243.0	0.35	45.8	4,700
22 July								
1	23.5	0.38	39.5	33.8	167.5	0.44	44.1	69,000
5	23.5	0.43	38.8	37.5	154.0	0.49	35.8	160,900
9	21.7	0.63	41.3	33.8	168.0	0.45	26.7	117,800
14	15.2	0.48	36.8	36.4	158.0	0.46	32.9	34,300
17	13.7	0.33	24.3	18.0	145.0	0.29	43.9	24,600
7 August								
1	23.8	0.65	72.0	41.5	200.5	0.57	30.9	96,400
5	23.7	0.75	60.0	47.5	217.5	0.49	29.0	159,300
9	22.6	0.65	70.0	45.5	210.0	0.55	32.3	113,700
14	15.6	0.58	45.5	22.5	216.5	0.31	37.3	39,500
17	13.6	0.70	29.3	26.5	191.0	0.29	27.3	10,700
12 August								
1	24.4	0.48	55.3	47.5	222.5	0.46	46.4	56,700
5	24.3	0.48	55.3	45.5	185.5	0.54	38.7	103,400
9	20.8	0.60	57.5	36.5	220.0	0.43	36.7	81,800
14	14.5	0.45	31.5	28.7	179.5	0.35	39.9	--
17	12.8	0.40	25.0	33.7	167.0	0.35	41.8	--

Table 2. continued

Depth (m)	Temp. (C)	Particulate matter (mg/L)	Carbohydrate fraction (ug/L)					Cellulose-containing phytoplankton (units/L)
			S	H	C	S+H/C	C as % dry wt	
14 August								
1	24.9	0.50	61.3	50.0	215.0	0.51	43.0	59,000
5	24.6	0.70	73.7	55.0	189.0	0.68	27.0	88,000
9	24.5	0.65	55.5	50.0	187.0	0.56	28.8	130,700
14	15.0	0.58	27.0	29.5	179.5	0.26	30.9	--
17	13.8	0.58	27.0	29.5	179.5	0.26	30.9	--
26 August								
1	--	0.88	60.5	37.0	188.5	0.52	21.4	--
5	--	0.93	58.7	30.0	189.0	0.47	20.3	--
9	--	1.03	50.0	62.5	216.5	0.52	21.0	--
14	--	0.58	39.0	31.2	199.0	0.35	34.3	--
17	--	0.80	25.0	21.2	210.0	0.22	26.3	--

Table 3. Distribution of selected parameters during summer stratification.

Station H

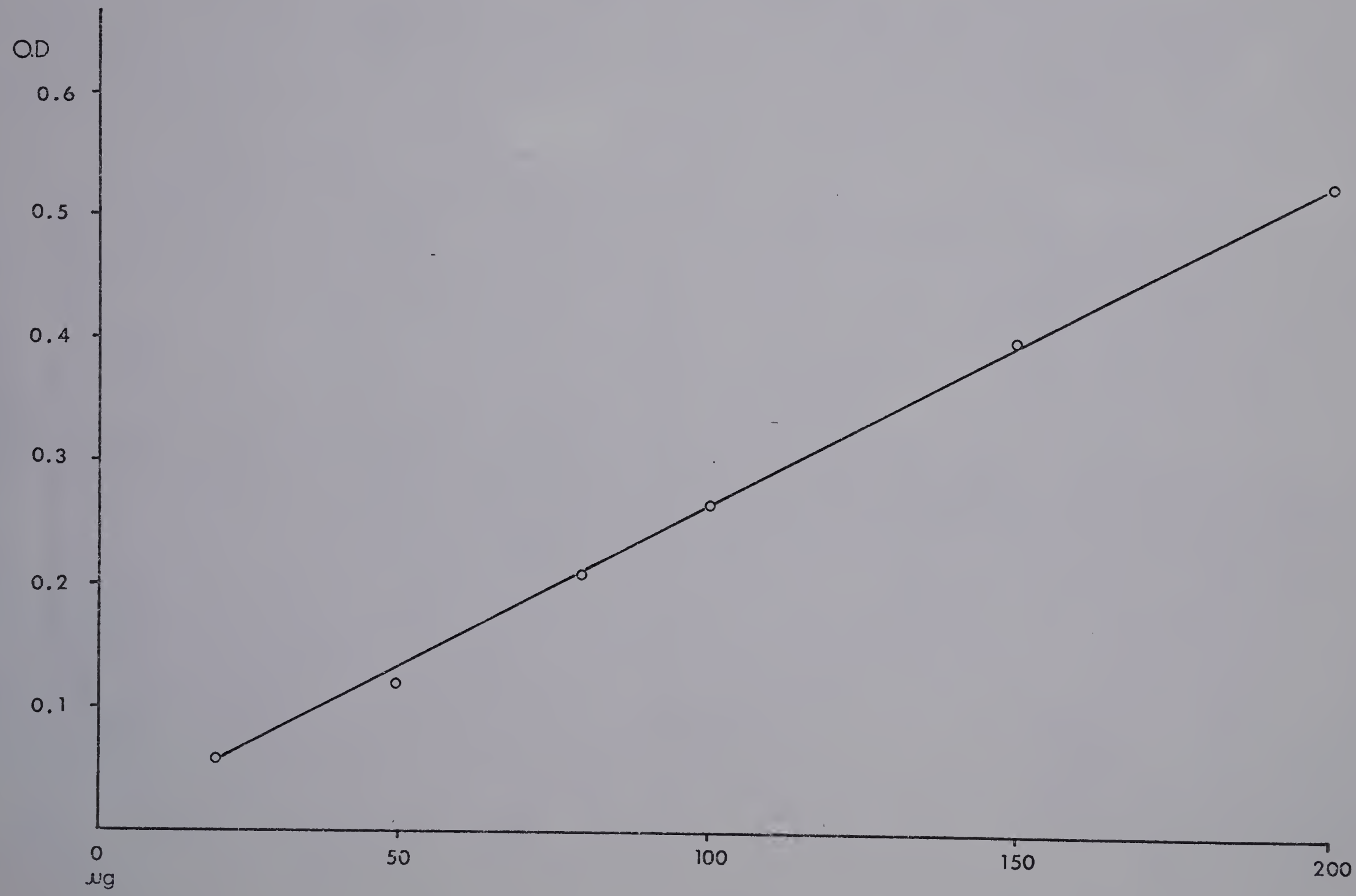
Depth (m)	Temp. (C)	Particulate matter (mg/L)	Carbohydrate fraction (ug/L)					Cellulose-containing phytoplankton (units/L)
			S	H	C	S+H/C	C as % dry wt.	
1 July								
1	24.5	1.02	63.3	44.7	114.7	0.94	11.2	—
4	24.4	0.77	54.3	35.0	165.3	0.54	21.4	—
8	17.8	1.03	65.5	92.1	197.9	0.79	19.3	—
12	14.5	2.80	96.5	133.5	363.0	0.63	13.0	—
16	14.3	6.60	165.0	112.5	710.0	0.40	10.7	—
17 July								
1	24.9	1.65	76.5	30.0	153.0	0.69	9.3	41,000
4	24.7	1.33	86.0	40.0	223.3	0.56	16.8	21,400
8	19.8	1.12	90.0	27.0	224.0	0.50	20.0	8,800
12	14.7	2.70	90.0	24.0	420.0	0.27	15.5	6,800 (debris)
16	13.4	22.71	407.1	96.4	1185.0	0.42	5.2	debris
31 July								
1	23.5	0.70	44.5	61.1	174.5	0.60	24.9	258,000
4	23.4	0.55	43.8	54.8	170.0	0.58	30.8	316,500
8	21.8	0.65	38.8	30.8	170.5	0.40	26.2	96,000
12	19.1	3.15	96.5	61.5	502.0	0.31	15.8	1,300
16	--	15.65	177.5	180.0	557.0	0.64	3.5	600 (debris)
19 August								
1		0.83	93.3	--	170.7	--	20.5	—
4		1.17	77.0	99.4	248.3	0.71	21.2	—
8		0.83	57.1	64.3	229.1	0.53	27.6	—
12		6.20	115.0	95.0	890.1	0.24	14.3	—
16		13.50	152.0	170.0	1140.1	0.28	8.4	—

APPENDIX II

Fig.1. Standard curve for glucose determination by phenol-sulfuric test.

APPENDIX II

Fig. 2. Standard curve for cellulose determination by anthrone reaction



APPENDIX III

Plate 1. Comparison of laboratory cultivated algae and natural environment grown algae.

A. Scenedesmus quadricauda (Indiana Univ. 76).

B. Scenedesmus sp. (Q) isolated from Quabbin Reservoir.

C. Scenedesmus sp. (L) isolated from Lower Pond.

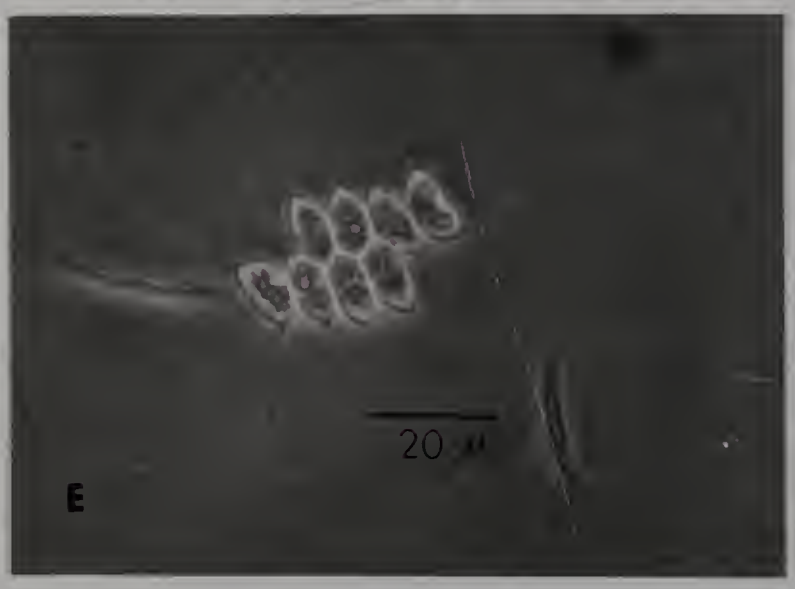
D-F. Scenedesmus sp. photographed directly from water samples.


A
12 μ


B
15 μ


10 μ
C


10 μ
D


20 μ
E


F

Plate 1. continued

G. Ankistrodesmus sp. isolated from Quabbin Reservoir.

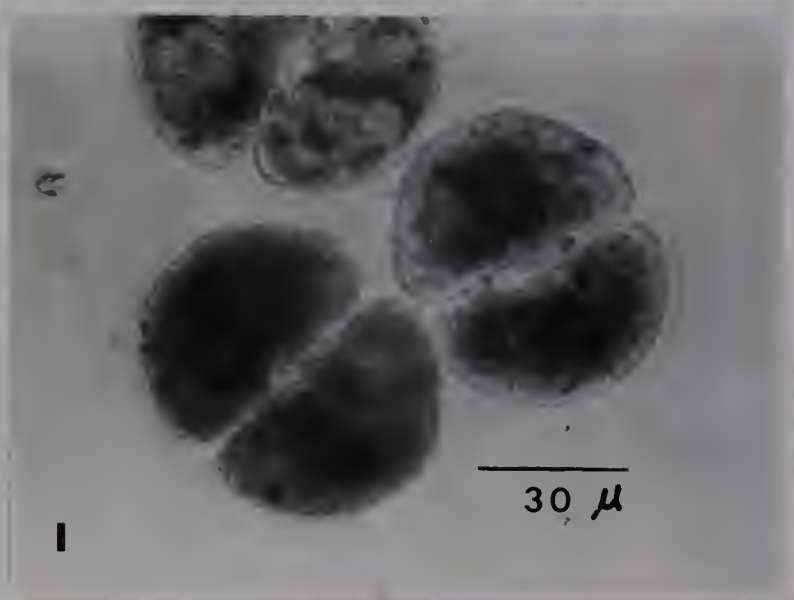
H. Ankistrodesmus sp. photographed directly from water samples.

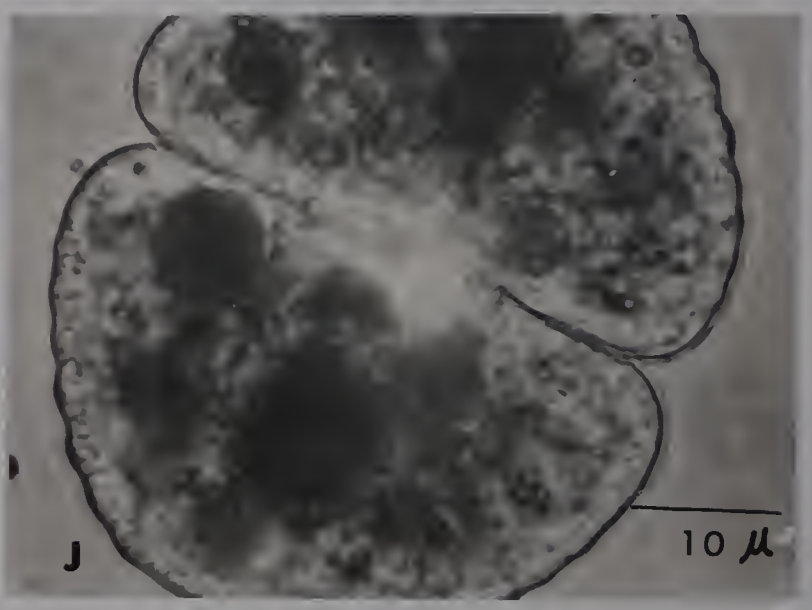
I, J. Cosmarium botrytis (Indiana Univ. 301).

K, L. Cosmarium sp. photographed directly from water samples.


G
10 μ


H
10 μ


30 μ
I


10 μ
J


10 μ
K


15 μ
L

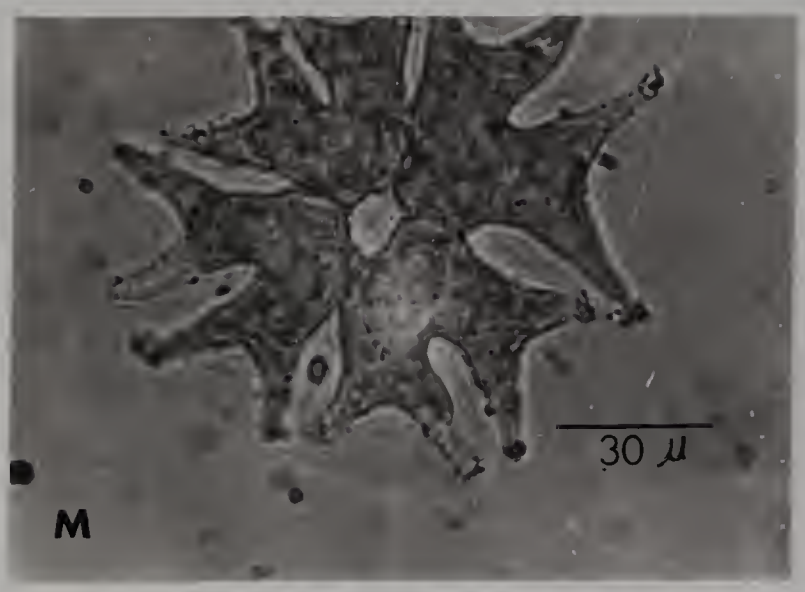
Plate 1. continued

M. Pediastrum bitradiatum (Indiana Univ. 37).

N-P. Pediastrum sp. photographed directly from water samples.

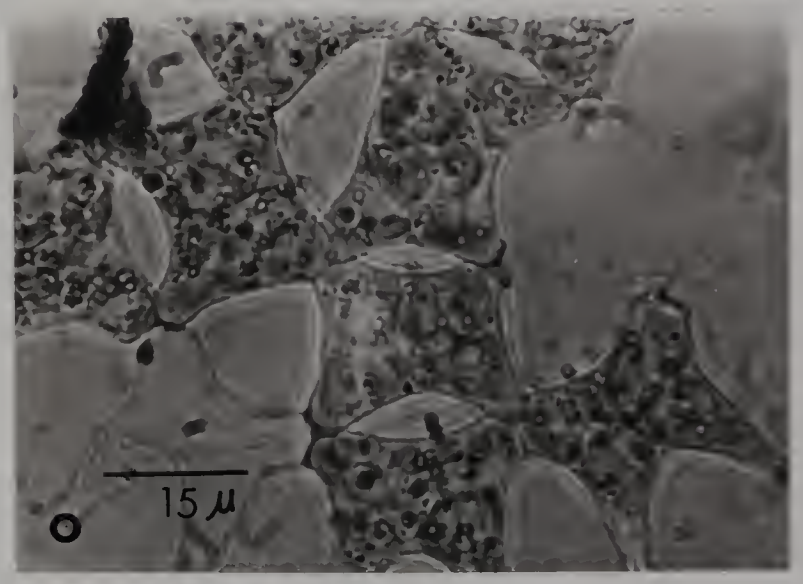
Q. Staurastrum orbiculare (Indiana Univ. 430).

R. Staurastrum sp. photographed directly from water samples.

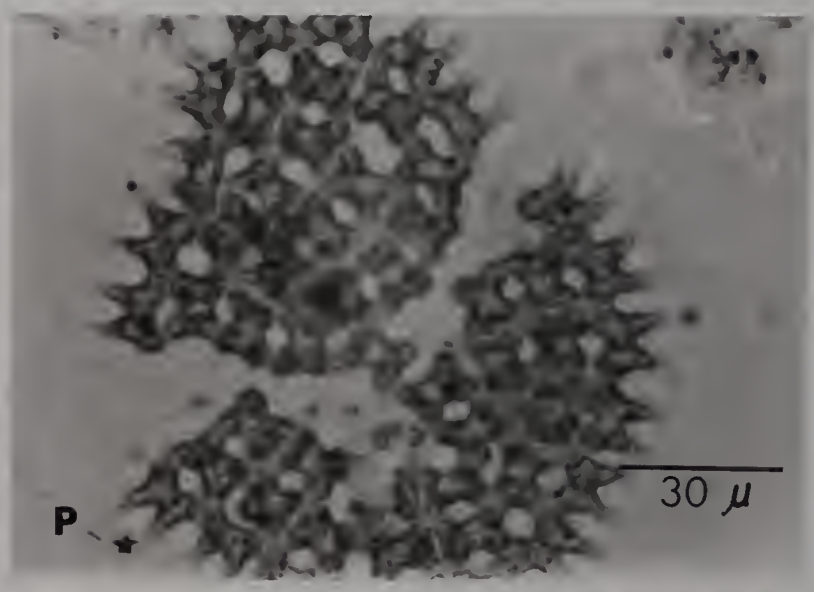


M

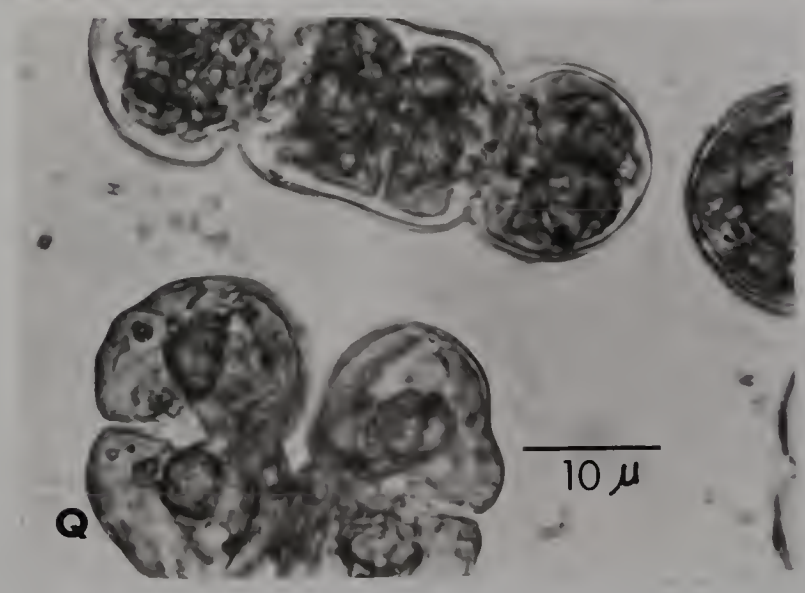
N



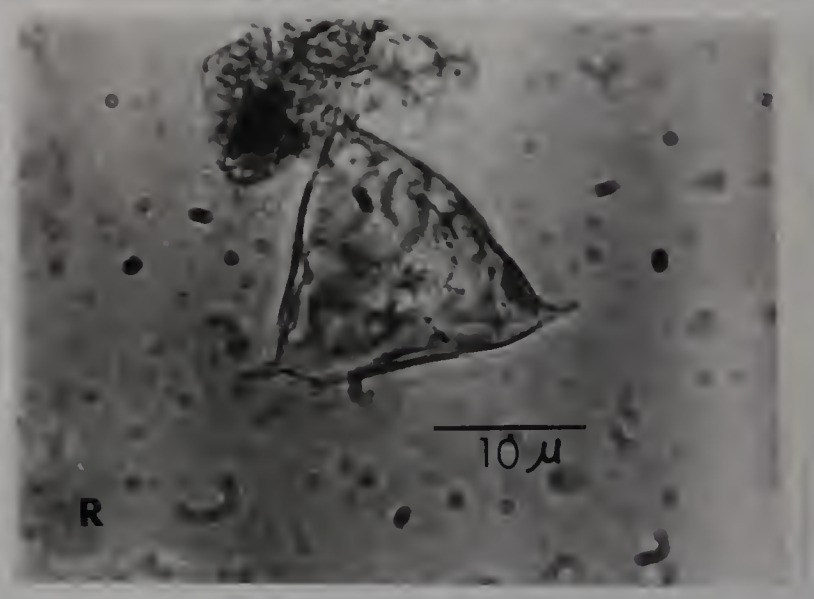
O



P



Q



R

APPENDIX III

Plate 2. Laboratory cultivated other algae.

a. Chlamydomonas sp. isolated from Lower Pond.

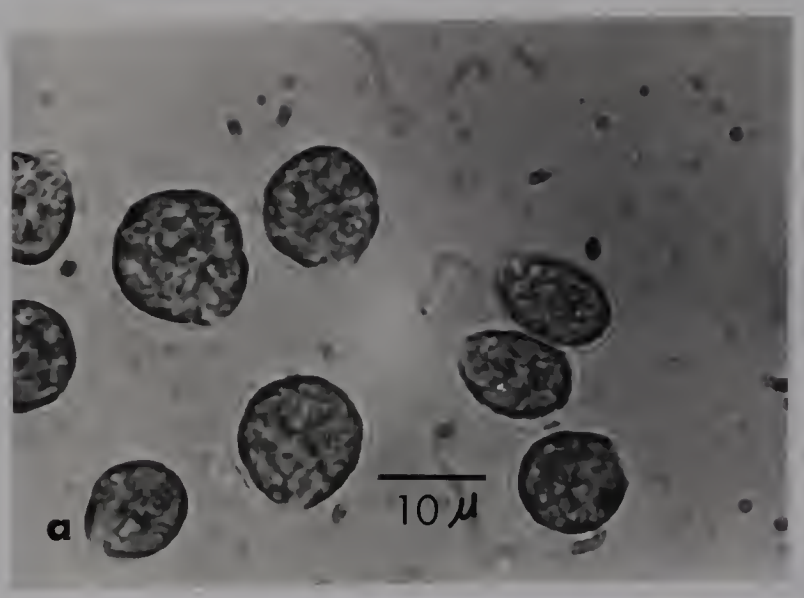
b. Chlorococcum sp. isolated from Quabbin Reservoir.

c. Oocystis marssonii (Indiana Univ. 287).

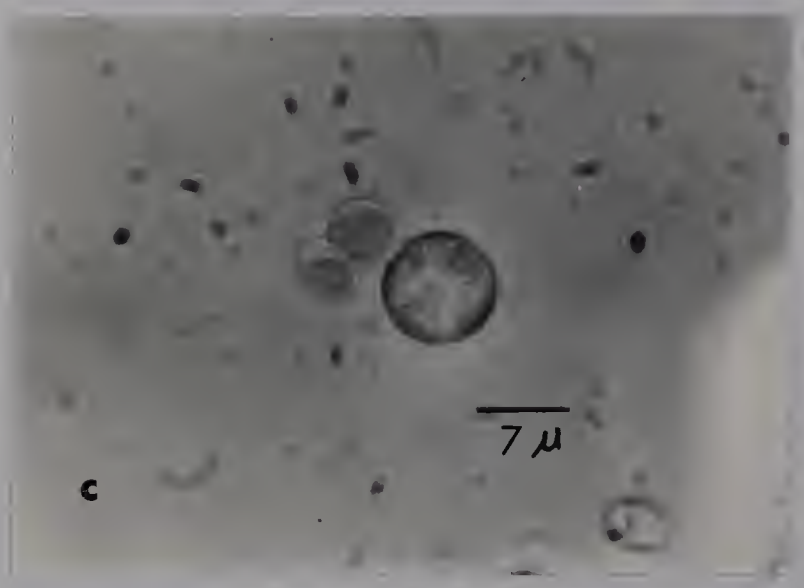
d. Gloeocystis sp. isolated from Quabbin Reservoir.

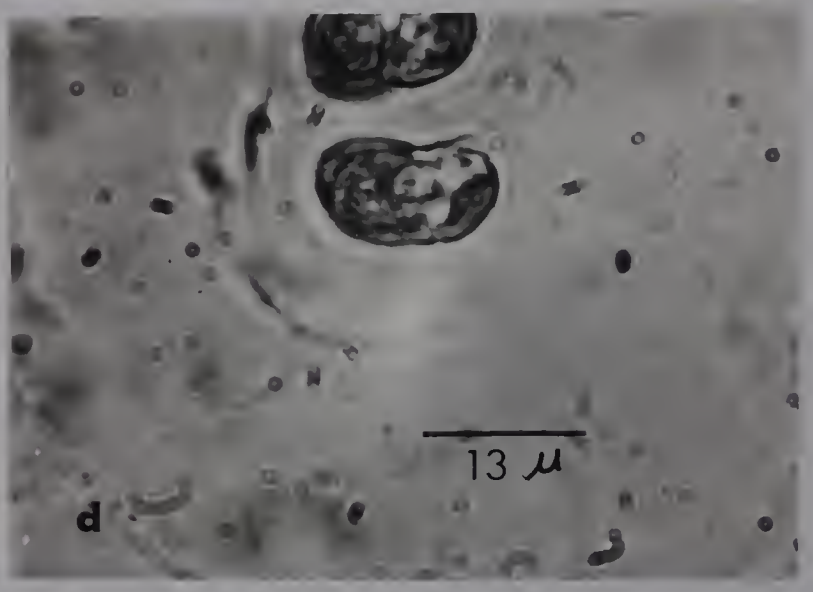
e. Coelastrum microporum (Indiana Univ. 281).

f. Tetraedron bitridens (Indiana Univ. 120).

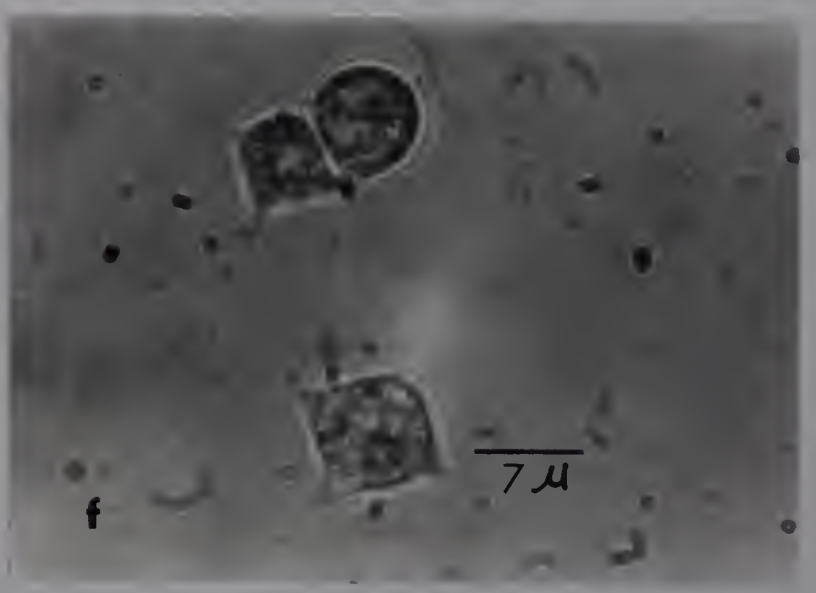

a
10 μ


b
13 μ


7 μ
c


13 μ
d


11 μ
e

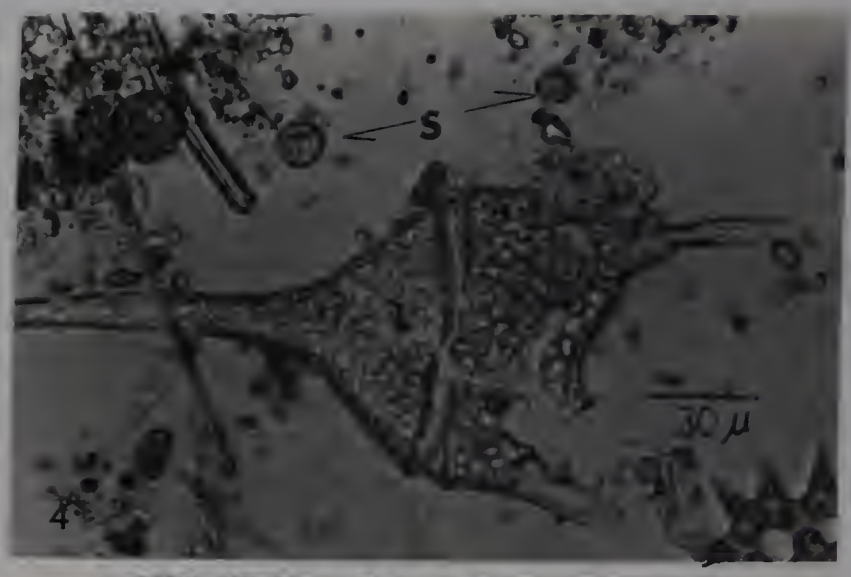

7 μ
f

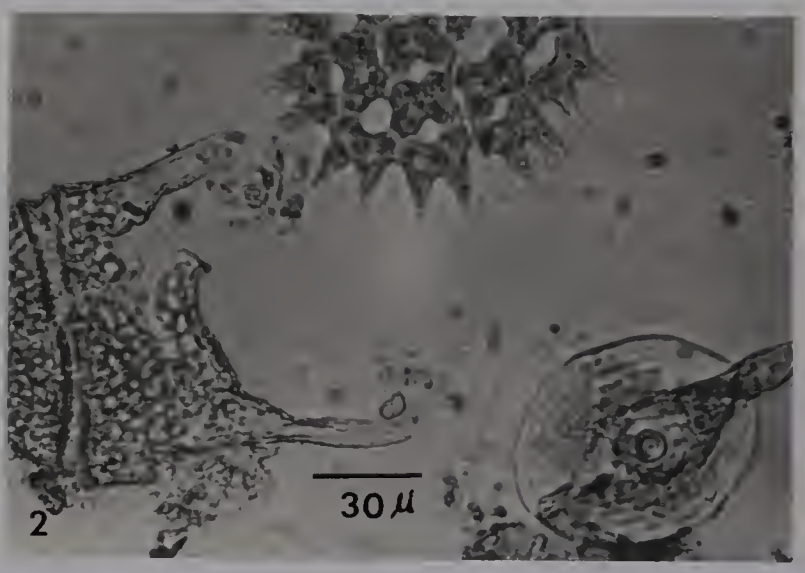
APPENDIX III

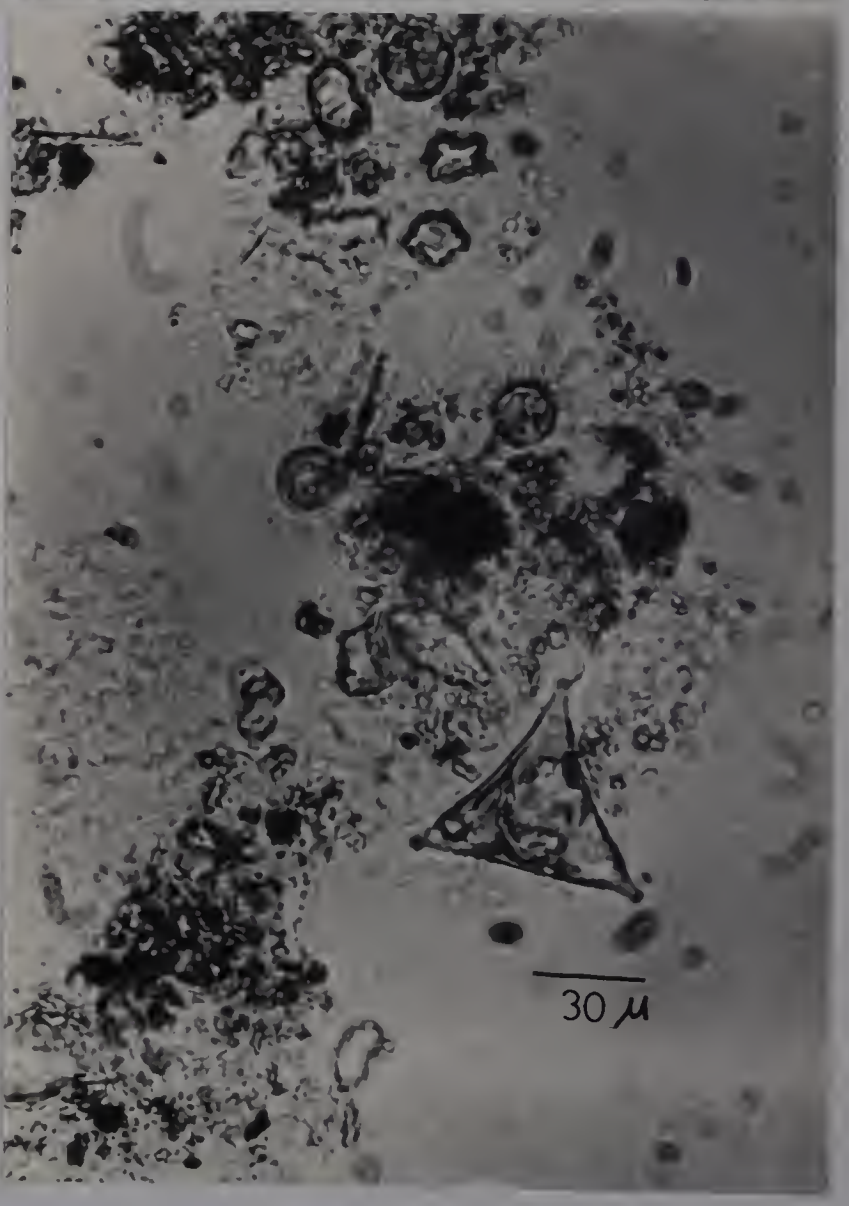
Plate 3. Natural environment grown other algae.

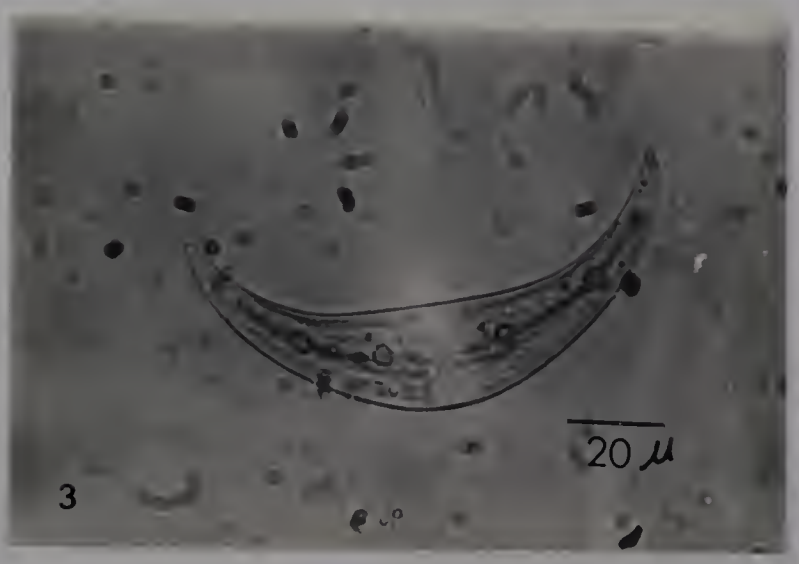
1. Golenkinia sp. photographed directly from water samples.
2. Ceratium sp., Phacus sp. and Pediastrum sp. photographed directly from water samples.
3. Closterium sp. photographed directly from water samples.
4. Size differences between larger and smaller algae.
5. Detritus made up of dead and living algae.


16 μ
1


S
30 μ
4


30 μ
2


30 μ


20 μ
3

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